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# BOVINE CONTAGIOUS ABORTION.

## THE USE OF GUINEA-PIGS IN IMMUNISATION STUDIES.

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THE few records of immunisation experiments against *Br. abortus* infection in guinea-pigs are insufficient to warrant any conclusions regarding the value of these animals for this type of work. Experiments with killed cultures have been recorded by Ascoli (1916), Stafseth (1920), Hagan (1922) and Gwatkins (1931). Ascoli and Stafseth concluded that no immunity was produced, but their methods of testing immunity were most probably too severe. Hagan paid adequate attention to experimental details but the numbers of animals used were small, only seven vaccinated animals being available for comparison with seven controls; he considered, however, that *Br. abortus* infection developed more slowly in the vaccinated animals. Gwatkins used repeated large inoculations of killed cultures but no immunity was demonstrated.

More significant results are reported from the inoculation of living cultures of low virulence. Huddleson (1924) stated that a living culture of a non-virulent strain of *Br. abortus* had been found to protect guinea-pigs from abortion, when exposed to infection by feeding the animals with virulent micro-organisms, but no details of his experiments are given. Schroeder and Cotton (1925) reported upon the inoculation of guinea-pigs with a strain which only caused lesions when very large doses were given. The strain, however, was recoverable from the spleens of guinea-pigs two months after their inoculation with moderate doses. They vaccinated guinea-pigs with heavy suspensions of this living culture and exposed some of these animals and control guinea-pigs to a subcutaneous infection with a bovine strain of *Br. abortus*; the other vaccinated animals, together with controls, were infected with a porcine strain of *Br. abortus*. The quantities of these

respective test inoculations are not given, but, on the whole, when the animals were killed and examined the vaccinated guinea-pigs showed fewer lesions or less extensive lesions than the controls. No bacteriological examinations were made.

Were it possible to increase the resistance of both guinea-pigs and cattle by the use of the same vaccine, the guinea-pig might prove a most valuable laboratory animal for the routine examination of vaccines used in the field.

The primary objective of the experiments now to be summarised was to ascertain whether a strain of *Br. abortus* of low virulence stimulated a measurable resistance to a subsequent exposure to an infection with a virulent strain of *Br. abortus*. Only one strain of low virulence has been tested, Strain No. 45. This strain was isolated some years ago in this country from material of bovine origin, most probably prior to 1922, but the exact date of its isolation is uncertain. It behaves as a typical bovine strain; it is agglutinated well by positive sera, and throughout the course of these experiments it has remained smooth, being heat-stable according to the criteria adopted by Pandit and Wilson (1932). Comparative tests have shown that the strain grows equally as well in ordinary atmospheric air as in an atmosphere containing 10 per cent. $CO_2$ gas, irrespective of whether the seed material is derived from a stock culture or from the tissues of an inoculated animal.

In 1931 a few preliminary experiments demonstrated the low virulence of the strain for guinea-pigs and suggested the possibility of its possessing antigenic value, and in September 1931 the current sub-culture of that date was arbitrarily designated Sub-culture X1, and sub-cultures from that time have been numbered in series.

Since March 1930, Strain 45 has been maintained exclusively on liver agar slants. The virulent strains used in testing the resistance or immunity of guinea-pigs have been grown exclusively on the same medium. All suspensions of the organisms used in the preparation of vaccine and for the test inoculations, etc., have been prepared from 48-hour growths on liver agar, washed off with normal saline solution and standardised to a required opacity, using Brown's opacity tubes for this purpose.

On a table supplied with the opacity tubes, the numbers of organisms in suspensions of *Br. melitensis* of opacity comparable to those of the different tubes in the set are given. It has been arbitrarily assumed that suspensions of *Br. abortus* contain a like number of micro-organisms to comparable suspensions of *Br. melitensis*. On this basis and accepting the numbers of *Br. melitensis* given in the above-mentioned table as correct, so in the succeeding records the numbers of *Br. abortus* in the different suspensions used are given, the reference to the actual opacity of the suspensions generally being omitted. Suspensions for the

agglutination test have been prepared either from Strain 45 or from another smooth strain. The same suspension has always been used throughout any one experiment.

In order to ensure a $CO_2$ sensitivity in the strain used to test for immunity it has been necessary to use more than one $CO_2$ sensitive strain throughout the experiments. In one experiment vaccinated animals were deliberately infected with a virulent ærobic strain.

Prior to 1933, when the experimental guinea-pigs were killed and examined, the spleen was removed and portions of the organ thoroughly teased with needles; a loopful of spleen tissue was then inoculated on to agar slants. When other tissues were examined for evidence of infection these were treated in a similar manner. From 1933 onwards the spleens were weighed and afterwards reduced to a pulp by scraping with a spatula; a quantity of the pulp was reserved for the inoculation of agar slants and the remainder of the pulp transferred to sterile thick-walled test tubes containing a number of glass beads, a measured volume of sterile saline solution was then added, the tube closed with a rubber stopper and vigorously shaken. A 1 : 10 dilution of the spleen pulp suspension was made in a second tube. From these two suspensions, amounts of 0·2 c.c. were sown on fuchsin agar plates, or on agar slants in large test tubes of 1 in. internal diameter and from the number of colonies which formed the number which theoretically could have been obtained from the whole spleen was estimated. From comparisons made on cultures from a large number of guinea-pigs, the enumeration of colonies obtained from spleen emulsions provided no more reliable data than that obtained by the direct inoculation of spleen pulp on agar.

When material from animals vaccinated with living cultures of Strain 45 was examined the absence of growth in atmospheric air was regarded as evidence of the elimination of the vaccine strain from the body.

Attempts have been made to passage Strain 45 through guinea-pigs. It was found impossible to passage the strain directly from animal to animal by the inoculation of spleen tissue, the strain invariably being lost in the second passage animal. The strain, however, was generally recoverable from the spleens of guinea-pigs inoculated seven to ten days previously with large numbers of bacteria, but growth was not abundant, only a few colonies being obtained per slant richly seeded with spleen pulp; subcultivation of these primary growths yielded rich cultures which were in turn inoculated into guinea-pigs, and so on. From February to May 1935, the strain was passaged through a series of six guinea-pigs by such means.

When Strain 45 was tested for pathogenicity in 1931 it was found that the subcutaneous or intraperitoneal inoculation of guinea-pigs each with 768 million micro-organisms or the

subcutaneous inoculation of twice that number failed to produce any macroscopic evidence of infection or to produce agglutinins in titres higher than 1 : 160. However, from the spleens of some animals a few colonies of *Br. abortus* were obtained even up to 33 days after inoculation. By June of 1934 such *in vivo* survival powers as the strain may have possessed three years previously had probably diminished, because the micro-organism was not recoverable after the fourth week in any one instance from the spleens of guinea-pigs inoculated subcutaneously with the relatively enormous dose of 61,480 million micro-organisms. The titre of the serum remained low, even after these large inoculations. In one experiment where 20 guinea-pigs were used, the serum from one animal agglutinated at a titre of 1 : 640 and the serum from another at 1 : 320, but the remaining sera all had lower titres.

The vaccination experiments of 1931 further indicated the absence of invasive properties of the strain even at that time, no evidence having been obtained from the examination of guinea-pigs in immunisation experiments of the persistence of the strain in the body of the guinea-pigs a few weeks after inoculation.

The conclusion that the strain was of low virulence for the guinea-pigs throughout the four years under consideration is justifiable. Furthermore, from the protocols of the last vaccination experiment which are summarised later, it is apparent that even after serial passage through six guinea-pigs the virulence of the passaged strain had not been exalted to an appreciable extent, if at all.

## Immunisation Experiments.

### 1931-32 *Experiments.*

Preliminary experiments made in 1931 indicated that guinea-pigs inoculated once with 1,537 million living micro-organisms of Strain 45 possessed greater resistance to infection caused by applying a drop of a suspension of a recently isolated $CO_2$ sensitive strain of *Br. abortus* containing 2,305 million organisms per cubic centimetre, to the eye, than control animals. Furthermore, the inoculation of three doses of living micro-organisms in ascending magnitude at approximately weekly intervals, stimulated an appreciable resistance in a group of guinea-pigs which included a number of female animals; and a comparison of pregnant vaccinated animals with pregnant control animals showed a greater susceptibility to uterine infection and abortion among the latter. The experiments of that year, however, indicated that when a number of guinea-pigs were inoculated with the same living vaccine and divided into two groups and later infected in a comparable manner but on different dates, the apparent resistance to infection of the groups might differ.

An opportunity to carry out further experiments did not occur until September 1932, when eleven male guinea-pigs each received

three subcutaneous inoculations, each consisting of 1,537 million organisms of Strain 45 Sub-culture X5. Fifty-nine days after receiving the final inoculation the vaccinated animals and an equal number of control male guinea-pigs received subcutaneously a test inoculation of a virulent $CO_2$ sensitive strain of *Br. abortus*. The test inoculation consisted of 1 c.c. of a suspension of organisms equivalent in opacity to No. 1 tube of the Brown scale (containing 768 million organisms per cubic centimetre) diluted $10^7$. The dilutions, however, were all made with the same pipette, and therefore the approximate number of organisms inoculated cannot be even roughly estimated. Equal numbers of the vaccinated and control animals were killed and examined later at intervals of 43, 57, 60 and 71 days respectively. None of the vaccinated animals showed lesions, whereas the characteristic splenic enlargement of a *Br. abortus* infection was shown by all the controls, and two of these animals, one killed on the forty-third day and the other on the sixtieth day, each showed abscessation of one testicle. *Br. abortus* was recovered in culture from the spleen of one vaccinated guinea-pig but was not obtained from the livers of any of the animals. *Br. abortus* was, on the other hand, recovered in culture from the spleens of all the control animals and from the livers of four of them. The titres of the sera when the animals were killed further reflected the decided resistance of the vaccinated animals to infection. The titres of vaccinated animals were 1 : 40, 1 : 80, 1 : 80, 1 : 40, 0, 1 : 80, 1 : 320, 0, 1 : 160, 0 and 1 : 80, and those of the control animals 1 : 640, 1 : 2,560, 1 : 2,560, 1 : 1,280, 1 : 10,240, 1 : 2,560, 1 : 640, 1 : 640, 1 : 80, 1 : 640 and 1 : 2,560.*

The results of this experiment were unequivocally in favour of the vaccinated group which showed, according to the criteria of the examination, a complete resistance in 90 per cent. of the animals to an exposure which caused infection in 100 per cent of the controls.

1933—*Experiment on Male Guinea-pigs.*

The next experiment was commenced in May 1933 and it was designed in the hope of repeating the favourable results of the preceding experiment : and also to try to compare the immunising properties of vaccines consisting of living organisms of Strain 45 Sub-culture X226, obtained by repeated sub-cultivation at intervals of one to two days; of heat killed suspensions of Strain 45 Sub-culture X9; and of formalin sterilised suspensions of the same strain and sub-culture, with suspensions of live organisms of the same.

Male guinea-pigs were used and vaccinated three times at intervals of 14 days. The animals given the living suspensions of *Br. abortus* received 3,074 million organisms at each inoculation;

* 0 = No agglutination at 1 : 20.

those receiving suspensions sterilised by heat and formalin respectively, 30,740 million organisms at each inoculation. The sterilisation of the former was carried out by heating in the water bath at 65° C. for one hour; the sterilisation by formalin was accomplished by adding sufficient formalin to the suspension of the organisms to give a final concentration of 0·5 per cent. formalin and incubating at 37° C. for 48 hours.

The vaccinated guinea-pigs and control animals each received a test inoculation 14 days after the administration of the last inoculation of vaccine. The test inoculation was similar to that employed in the preceding experiment of September 1932, except in so far as the sixth instead of the fourth sub-culture of the $CO_2$ sensitive strain was used. The guinea-pigs were killed and examined 31, 34, 35, 39, 40, 45, 48 and 49 days after infection, as far as possible equal numbers being killed from each group at the same time.

The results of this experiment were not very satisfactory and may be summarised as follows: Of 15 animals inoculated with live vaccine from Sub-culture X9, eleven, or 73 per cent., were infected, and one, or 6 per cent., showed macroscopic lesions; of 13 animals inoculated with live vaccine from Sub-culture X226, ten, or 77 per cent., were found to be infected, and two, or 15 per cent., showed macroscopic lesions; of 15 animals inoculated with heat killed vaccine, fourteen, or 93 per cent., were infected, and eight, or 53 per cent., showed macroscopic lesions; of 15 inoculated with formalinised vaccine, twelve, or 80 per cent., were infected, and four, or 26 per cent., showed macroscopic lesions; 16 out of 16 controls were infected, and six, or 37 per cent., showed macroscopic lesions.

The experiment, though of limited value, does not suggest that the repeated sub-cultivation had depleted Strain 45 of antigenic properties and it indicates that heat killed vaccine may be of little value.

In a further experiment in 1933 comparisons were made between male guinea-pigs inoculated four times with 61,840 million live organisms at intervals of one week and similar animals inoculated in the same manner but with a number of micro-organisms ten times smaller. The animals were infected six weeks after the last inoculation of vaccine. Owing to intercurrent disease the numbers of the animals in experiment were considerably reduced but the summarised results in Table I show that vaccination with either the large or the smaller dose had increased the animals' resistance to infection.

1934—*Experiments on Male Guinea-pigs.*

An experiment was commenced in May 1934, and was designed to test the effect of large multiple doses of vaccine; multiple smaller sized doses; a single large dose and single smaller dose;

| | *No. of G. Pigs per group.* | *No. and Percentage found infected.* | *No. and Percentage showing Macroscopic Lesions.* | *Average No. of Colonies from Spleen Cultures, Infected Animals only considered.* | *No. and Percentage with infected Livers.* | *Average No. of Colonies from Liver Cultures.* | *Average Titre of Serum.* |
|---|---|---|---|---|---|---|---|
| Group 1. Large multiple inoculations | 10 | 2<br>20 per cent. | 0<br>0 per cent. | 7 | 0<br>0 per cent. | 0 | 25 |
| Group 2. Moderate multiple inoculations | 6 | 2<br>33 per cent. | 0<br>0 per cent. | 8 | 0<br>0 per cent. | 0 | 38 |
| Group 5. Controls ... | 8 | 8<br>100 per cent. | 8<br>100 per cent. | 104 | 3<br>37 per cent. | 3 | 640 |

In estimating the average titre of the serum, the dilution of the serum at the end point of agglutination was regarded as a fraction.

## TABLE II.

PLAN OF EXPERIMENT OF MAY, 1934.

| *Group of Guinea Pigs.* | *Weeks.* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *1st.* | *2nd.* | *3rd.* | *4th.* | *5th.* | *6th.* | *7th.* | *8th.* | *9th.* | *10th.* | *15th.* | *20th.* | *25th.* |
| 1 ... | 10uk. | 10uk. | 10uk. | 10uk. | | | | | | Inf. | Ex. | | |
| 2 ... | 1ul. | 1ul. | 1ul. | 1ul. | | | | | | ,, | ,, | | |
| 3 ... | 10ul. | 10ul. | 10ul. | 10ul. | | | | | | ,, | ,, | | |
| 4 ... | | | | 1ul. | | | | | | ,, | ,, | | |
| 5 ... | | | | 1ul. | | | | | | ,, | ,, | | |
| 6 ... | | | | | | 1ul. | 1ul. | 1ul. | 1ul. | ,, | ,, | | |
| 7 ... | | | | | | 10ul. | 10ul. | 10ul. | 10ul. | ,, | ,, | | |
| 8 ... | | | | | | | | | 1ul. | ,, | ,, | | |
| 9 ... | | | | | | | | | 10ul. | ,, | ,, | | |
| 10 ... | | | | | | | | | | ,, | ,, | | |
| 11 ... | | 1ul. | 1ul. | 1ul. | 1ul. | | | | | | | Inf. | Ex. |
| 12 ... | | 10ul. | 10ul. | 10ul. | 10ul. | | | | | | | ,, | ,, |
| 13 ... | | | | | | | | | | | | ,, | ,, |

10uk. = 10 units of killed vaccine, one unit containing 6,148 million micro-organisms.
1ul. = 1 unit of living vaccine, one unit containing 6,148 million micro-organisms.
10ul. = 10 units of living vaccine, one unit containing 6,148 million micro-organisms.
Inf. = Infected with 23,000 organisms of a virulent $CO_2$ sensitive strain of *Br. abortus*. In preparing these and all subsequent test inoculations a separate pipette was used in making each dilution.
Ex. = Killed and examined.

TABLE III.

| *Group and Vaccine.* | *Dose of Vaccine.* | *Time in Weeks before Infection.* | *No. of G. Pigs per Group.* | *No. and Percentage Infected.* | *No. and Percentage Heavily Infected in Spleen.* | *No. and Percentage Infected in Liver.* | *Average Weights of Spleens.* | *No. of Sera with a Titre of 1 : 10 and Higher and Average Titre of these Sera.* |
|---|---|---|---|---|---|---|---|---|
| 1. Heat killed | L.M. | 6 | 15 | 12<br>80 per cent. | 6<br>40 per cent. | 1<br>6·6 per cent. | 1·06 | 14<br>56 |
| 2. Living | S.M. | 6 | 17 | 1<br>5·9 per cent. | 0<br>0 per cent. | 0<br>0 per cent. | 0·77 | 16<br>32 |
| 3. Living | L.M. | 6 | 16 | 4<br>25 per cent. | 0<br>0 per cent. | 0<br>0 per cent. | 0·84 | 13<br>29 |
| 4. Living | S.S. | 6 | 18 | 3<br>16·6 per cent. | 0<br>0 per cent. | 0<br>0 per cent. | 1·00 | 14<br>34 |
| 5. Living | L.S. | 6 | 18 | 3<br>16·6 per cent. | 0<br>0 per cent. | 0<br>0 per cent. | 0·85 | 17<br>41 |
| 6. Living | S.M. | 1 | 18 | 18<br>100 per cent. | 2<br>11 per cent. | 1<br>5·5 per cent. | 0·97 | 18<br>134 |
| 7. Living | L.M. | 1 | 17 | 15<br>88·2 per cent. | 1<br>5·8 per cent. | 1<br>5·8 per cent. | 0·89 | 16<br>117 |
| 8. Living | S.S. | 1 | 17 | 14<br>82·3 per cent. | 1<br>5·8 per cent. | 1<br>5·8 per cent. | 0·89 | 16<br>50 |
| 9. Living | L.S. | 1 | 17 | 12<br>70·5 per cent. | 3<br>17·4 per cent. | 3<br>17·4 per cent. | 0·93 | 17<br>45 |
| 10. Controls | — | — | 18 | 18<br>100 per cent. | 8<br>44 per cent. | 7<br>38·5 per cent. | 1·36 | 18<br>147 |
| 11. Living | S.M. | 15 | 18 | 17<br>94 per cent. | 2<br>11·5 per cent. | 3<br>16·5 per cent. | 1·45 | 18<br>241 |
| 12. Living | L.M. | 15 | 16 | 13<br>71·1 per cent. | 2<br>12·4 per cent. | 2<br>2·4 per cent. | 1·11 | 16<br>106 |
| 13. Controls | — | — | 18 | 18<br>100 per cent. | 6<br>33·3 per cent. | 8<br>44 per cent. | 1·88 | 18<br>461 |

Aerobic strains of *Br. abortus* were isolated from five animals in each of Groups 6 and 7 and from 6 animals in each of Groups 8 and 9 indicating that the vaccine strain had not been eliminated from the body. Ordinarily the vaccine strain is not recoverable from the tissues 6 weeks after vaccination, and its isolation from the animals in these groups must be attributed to the effect of inoculating the animals with a virulent culture shortly after they had been vaccinated.

L.M. = large multiple : S.M. = small multiple : L.S. = large single : S.S. = small single.

and also to test the immunity at different periods of time after the last inoculation of vaccine. Furthermore, it was planned to test a vaccine composed of dead organisms sterilised by heat. The living vaccine was in each case prepared from Strain 45 Sub-culture X14; the dead vaccine was prepared from the same strain Sub-culture X13. The vaccine sterilised by heat had been maintained at a temperature of 60° C. for one hour, no preservative was added, and the vaccine was kept in the ice chest.

The plan of the experiment and its results are given in Tables II and III.

From the above summary it is evident that where the living vaccine was used satisfactory results were obtained with either multiple or single inoculations of large or smaller doses of vaccine when the test inoculation was given six weeks after the last inoculation of vaccine, the best results being shown by Groups 2, 3, 4 and 5. When the results of these groups are considered together they show that out of 69 guinea-pigs 58, or 84 per cent., completely resisted an infection which was sufficient to infect all of the 18 control animals.

When the test inoculation was given one week after the inoculation of the last dose of vaccine the results were not satisfactory, but in none of these vaccinated groups is there any evidence that the inoculation, even of large quantities of the vaccine strain only one week before infection, had increased the susceptibility to infection. Indeed, the evidence indicates some slight increase in resistance, as nine out of 69 guinea-pigs in Groups 6, 7, 8 and 9 resisted infection. Furthermore, in some of the remaining 60 guinea-pigs it is probable that the infection which was found was due to a persistence of the vaccine strain.

In this experiment those animals which received one inoculation only of vaccine, Groups 8 and 9, and could have developed no immunity from previous inoculations of vaccine, were significantly no more susceptible than the controls, Group 10, although viable organisms from the vaccine must have been present in the bodies of the vaccinated animals when the test inoculation was administered.

In that portion of the experiment where the resistance of the animals was tested 15 weeks after the last inoculation of vaccine the results are interesting. Although the animals in Groups 11 and 12 were but very little more resistant than the controls, they had been inoculated with comparable and in some instances with exactly the same vaccine which had produced a very definite immunity in the majority of the guinea-pigs in Groups 2, 3, 4 and 5. Therefore the failure to demonstrate a definitely increased resistance in the animals in Groups 11 and 12 cannot be attributed to any variation in the vaccine. Groups 11 and 12 were infected 15 weeks after vaccination, whereas Groups 2, 3, 4 and 5 were exposed to infection only six weeks after vaccination, but it is

most unlikely that a detectable diminution in immunity could occur between the seventh and the sixteenth week following vaccination. Moreover, in a later experiment immunity was shown to persist for at least 19 weeks. In the present experiment the same virulent strain of *Br. abortus* was used in the preparation of the test inoculations for all the groups of guinea-pigs, and when test inoculations were prepared comparable methods were employed in standardising the suspensions of culture to a like capacity and in the preparation of the requisite dilutions. Nevertheless it is probable that the inconstant results of this and of some other experiments were due to a variation in the severity of the test inoculations used and that these variations were uncontrollable by the methods employed.

The object of the next experiment was to test the effect of exposing vaccinated guinea-pigs to an infection with a virulent ærobic strain of *Br. abortus* and later exposing the animals to a second infection, but with a $CO_2$ sensitive strain of *Br. abortus*. Furthermore, the experiment was so arranged that it might furnish some data upon the duration of increased resistance in guinea-pigs.

Three groups of vaccinated and three groups of control guinea-pigs were used. The vaccinated groups are designated V.1, V.2 and V.3, and the control groups C.1, C.2 and C.3. All the animals in the vaccinated groups were inoculated at the same time and with the same vaccine, each animal receiving one inoculation of 6,148 million live organisms of Strain 45, Sub-culture X13.

Eight weeks after vaccination the animals in Groups V.1 and V.2, and the control guinea-pigs in Group C.1, were each inoculated with 30,700 organisms of a virulent ærobic strain of *Br. abortus*. Nineteen weeks after receiving this test inoculation the animals in Groups V.1 and C.1 were killed and examined. *Br. abortus* was isolated from three of the vaccinated animals, in two instances in very small numbers only, and from eight of the ten control guinea-pigs, thereby indicating that the vaccinated animals had possessed an increased resistance to infection. The guinea-pigs in Groups V.3 and C.3 were each inoculated with 30,700 organisms of a $CO_2$ sensitive strain 19 weeks after the animals in Group V.3 had been vaccinated. Seven weeks later the animals in both of these groups were killed and examined, that is on the twenty-sixth week of the experiment. Of the nine surviving vaccinated animals none was found infected with *Br. abortus*, whereas this micro-organism was recovered from eight out of ten animals in the control group. This portion of the experiment therefore furnished evidence that an immunity produced by vaccination was present 19 weeks after vaccination. The vaccinated animals in Group V.2 which had been inoculated eight weeks after vaccination with the virulent ærobic strain of *Br. abortus* were each inoculated 18 weeks later, that is 26 weeks after vaccina-

tion, with 30,700 organisms of the $CO_2$ sensitive strain previously used for the inoculation of Groups V.3 and C.3; the control animals in Group C.2 were similarly inoculated at this time. Six weeks later the animals in Groups V.2 and C.2 were killed and examined. *Br. abortus* was recovered from three of the nine surviving animals in Group V.2 and in each instance the recorded organisms were ærobic. All of the eight surviving control animals in Group C.2 showed a heavy infection with a $CO_2$ sensitive strain of *Br. abortus*.

In considering the results obtained in the six groups it appears that in Groups V.1 and C.1 the vaccination had produced a partial immunity to the inoculation of the ærobic strain of *Br. abortus* eight weeks later, and in Groups V.3 and C.3 a definite immunity to the inoculation of the $CO_2$ sensitive strain 19 weeks after vaccination. Furthermore, that vaccinated guinea-pigs exposed to infection with the ærobic strain, Group V.2, were able to withstand a later exposure to a $CO_2$ sensitive strain although certain of the guinea-pigs were at the time actually infected. The resistance of these guinea-pigs to the superimposition of infection is of particular interest and the experiment indicates the feasibility of testing the immunity produced by exposure to ærobic strains of some degree of pathogenicity, either in previously vaccinated animals or in normal animals, when the final test for increased resistance is carried out with a $CO_2$ sensitive strain. Finally, the experiment showed that an immunity response to the inoculation of the non-virulent Strain 45 persisted for a period of at least 19 weeks.

A summary of the experiment is given in Table IV.

TABLE IV.

| *Groups of G. Pigs.* | *Weeks of Experiment.* | | | | *No. of G. Pigs Examined.* | *Percentage found Infected.* |
|---|---|---|---|---|---|---|
| | *8th.* | *19th.* | *26th.* | *32nd.* | | |
| Vaccinated 1 | Inf. with ærobic | Examine | | | 10 | 30 with ærobic. |
| Control 1 | „ | „ | | | 10 | 80 with ærobic. |
| Vaccinated 2 | „ | | Inf. with $CO_2$ | Examine | 9 | 33 with ærobic only. |
| Control 2 | | | „ | „ | 8 | 100 with $CO_2$. |
| Vaccinated 3 | | Inf. with $CO_2$ | Examine | | 9 | None. |
| Control 3 | | „ | „ | | 10 | 80 with $CO_2$. |

1934—*Experiments on Female Guinea-pigs.*

It had been noted during 1931 that infected pregnant female guinea-pigs showed a heavy *Br. abortus* infection of the uterus; and that vaccinated pregnant guinea-pigs after exposure to infection, though sometimes infected in the spleen, might be free from

a uterine infection, or less severely infected in that organ than comparable control animals. If by vaccination with a non-virulent strain of *Br. abortus* it were possible to increase the resistance of the pregnant uterus to infection, even although the immunity were insufficient to afford the guinea-pig complete protection against infection, it would encourage the hope that the comparable treatment of the bovine might give equally favourable results in the protection of the pregnant uterus.

In the experiment about to be described female guinea-pigs were used and were infected after mating. Although abortion in infected guinea-pigs has been observed to occur with considerable regularity, nevertheless it was difficult to detect in individuals kept in groups, therefore reliance had to be placed on evidence of uterine infection at *post-mortem* examination, and the guinea-pigs were killed when it was considered most likely to find the majority of the animals pregnant or to have but recently aborted or given birth to young. Infection of the pregnant uterus was demonstrated by inoculating portions of the placentæ on to liver agar slopes. In cases of uterine infection this seed material affords luxuriant growths of *Br. abortus*, the organisms, indeed, may frequently be demonstrated in large numbers in suitably stained smears made from the tissues at the apposition of the fœtal and maternal discs. Following the abortion, or possibly the parturition, of an infected guinea-pig, single or multiple abscessation, with honeycombing and thickening of the uterine wall in the neighbourhood of the abscessation, frequently occurs. These lesions have been observed exclusively in *Br. abortus* infected animals, and in many cases the thickish cream-coloured pus from the abscesses yields abundant and pure cultures of *Br. abortus*, but at other times media inoculated with material from the uterine lesions is overgrown by contaminating bacteria.

Two groups of virgin female guinea-pigs were vaccinated, the one, Group (*a*), on March 21st, 1934; and the other, Group (*b*), on June 11th, 1934. The animals in both groups were each inoculated with 61,470 million organisms of Strain 45, those inoculated into Group (*a*) being from Sub-culture X15 and those used for the inoculation of Group (*b*) from Sub-culture X17. On June 11th a suitable number of male animals were introduced into each vaccinated group and also into a control group of normal females, Group (*c*). On July 2nd, 1934, all the female guinea-pigs received one drop on the eye of a saline suspension of a $CO_2$ sensitive strain of *Br. abortus* containing approximately 2,000 million bacteria per cubic centimetre. These animals were again infected in the same manner by applying a similar suspension to the opposite eye on July 5th. This double test dose of a virulent organism was very severe, experiments having demonstrated that very dilute suspensions of *Br. abortus*, when instilled on to the eye may set up infection with regularity. The animals

were killed from the twenty-eighth to the thirty-sixth day after first being infected, there being twelve females in Group (*a*), eleven in Group (*b*) and 21 in the control group (*c*). Unfortunately bacteriological examinations were made on only ten animals from Group (*a*), nine from Group (*b*) and 18 from the control group (*c*).

These bacteriological examinations showed that all the guinea-pigs were infected but the different groups were not affected to a like degree, the disease being mildest in Group (*a*) and most severe among the controls. Thus the average spleen weights were: Group (*a*) 1·72 grammes, Group (*b*) 2·27 grammes, and Group (*c*) 3·06 grammes, and the average estimated number of colonies per spleen was: Group (*a*) 9,600, Group (*b*) 61,800, and Group (*c*) 331,000. The superiority of the animals in Group (*a*) was maintained in the bacteriological examination of the livers and uteri. The examinations of the former organ showed none infected from Group (*a*); while four out of nine from Group (*b*) and 14 out of 18 in Group (*c*) were infected. The examinations of the uteri were particularly interesting and are summarised in Table V.

TABLE V.

| *Condition of Uterus.* | | | *Cultures made from the Uterus or Placentae.* | | |
|---|---|---|---|---|---|
| *Groups.* | | | *Groups.* | | |
| *A.* | *B.* | *C.* | *A.* | *B.* | *C.* |
| P. | P. | P. | ——— | None made | ——— |
| P. | P. | Abt. | | „ „ | |
| | | P. | | „ „ | |
| P. | Abs. | P. | Neg. | Contin. | Contin. |
| P. | P. | P. | „ | Neg. | „ |
| P. | | P. | Contin. | | „ |
| P. | | P. | Neg. | | „ |
| | | Not P.N. | | | Neg. |
| | | „ | | | „ |
| P. | Abs. | „ | Neg. | Contin. | „ |
| | | P. | | | Contin. |
| | | P. | | | „ |
| Abs. | Abs. | Abs. | O.B. | O.B. | O.B. |
| P. | Abs. | P. | Neg. | O.B. | Contin. |
| Not P.N. | P. | E.P. | „ | Neg. | „ |
| P. | P. | Abs. | „ | Contin. | „ |
| E.P. | P. | Abs. | „ | „ | „ |
| | Not P.N. | Not P.N. | | Neg. | Neg. |
| | | „ | | | „ |
| | | E.P. | | | „ |
| | | Abs. | | | Contin. |

P. = Pregnant; E.P. = Early pregnancy; Abt. = Abortion; Abs. = Abscessation of uterus; Not P.N. = Not pregnant, uterus normal.

Neg. = No bacterial growth; Contin. = Continuous growth of *Br. abortus* on surface of agar; O.B. = No growth of *Br. abortus* but a growth of other bacteria.

Considering those animals examined bacteriologically, it is seen that seven animals in Group (*a*) were well advanced in

pregnancy, and in only one of these was the uterus found infected. One animal in Group (*a*) showed abscessation of the uterus and one was in an early stage of pregnancy. *Br. abortus* was not isolated from the uterus of either of these animals but the abscessation is regarded as the direct sequel to *Br. abortus* infection. Therefore of the guinea-pigs which were pregnant or showed evidence of having been pregnant, only two out of nine, or 22 per cent., were infected or had suffered from a uterine infection with *Br. abortus*.

Seven control guinea-pigs were well advanced in pregnancy and all were infected in the uterus; four were suffering from abscessation of the uterus; two were in early pregnancy and one of these was infected. Therefore, out of 13 guinea-pigs which were pregnant or showed evidence of having been pregnant twelve, or 92 per cent., were, or had been, infected in the uterus with *Br. abortus*.

The animals in Group (*b*) were little, if at all, more resistant to uterine infection than the controls. The unsatisfactory results shown by Group (*b*) might be attributable to the use of a deteriorated vaccine. The only difference between the vaccines was that the vaccine inoculated into Group (*b*) animals was prepared from a culture two sub-cultures removed from that which provided the vaccine for Group (*a*), but it is unlikely that this was responsible for a deterioration of the vaccine.

The experiment in general confirmed the impression formed in 1931 that a considerable resistance to uterine infection could be induced by immunisation.

The next experiment was commenced in November 1934, its chief purpose being to obtain further information regarding the resistance of vaccinated guinea-pigs to *Br. abortus* infection acquired during pregnancy; both living and formalinised vaccines were tested.

Forty-eight female guinea-pigs believed to be non-pregnant were selected and divided into six groups. These animals were kept under observation for eight weeks prior to any inoculations being made and at the end of that period used for experiment. The groups were then treated as follows:—

*Group* 1.—Inoculated subcutaneously once with 6,148 million live organisms of Strain 45, Sub-culture X14.

*Group* 2 (six guinea-pigs).—Inoculated subcutaneously with 30 million live organisms, Strain 45, Sub-culture X14.

*Group* 3.—Inoculated subcutaneously with 30,740 million dead organisms, Strain 45, Sub-culture X14, formalinised vaccine.

*Group* 4.—Inoculated subcutaneously with 30 million dead organisms, Strain 45, Sub-culture X14, formalinised vaccine.

*Group* 5.—Inoculated subcutaneously with 30,740 million dead organisms of a virulent $CO_2$ sensitive strain No. 253, formalinised vaccine, prepared from Sub-culture 10.

*Group* 6.—Controls.

Four weeks after vaccination two male guinea-pigs were placed with each group of females and two weeks later the females each received a subcutaneous inoculation of what was estimated to be 30,700 live abortion bacteria of Strain 253, Sub-culture 12; the guinea-pigs were killed and examined 44 and 58 days later. The results of these examinations are shown in Table VI.

TABLE VI.

| | | |
|---|---|---|
| Group 1. | Live vaccine, moderate dose. | |
| | Titres of sera after vaccination and before infection : | 1 : 80–1 : 320. |
| | No. of guinea-pigs examined, 8. | |
| | „ „ found pregnant, 6. | |
| | „ „ infected in the uterus, 0. | |
| Group 2. | Live vaccine, small dose. | |
| | Titres of sera after vaccination and before infection : | Negative at 1 : 10–1 : 160. |
| | No. of guinea-pigs examined, 6. | |
| | „ „ found pregnant, 1. | |
| | „ „ infected in the uterus, 1. | |
| Group 3. | Live vaccine strain sterilised by formalin. | |
| | Titres of sera after vaccination and before infection : | Negative at 1 : 10–1 : 40. |
| | No. of guinea-pigs examined, 8. | |
| | „ „ found pregnant, 1 (dead fœtus). | |
| | „ „ infected in the uterus, 6. | |
| Group 4. | Live vaccine strain sterilised by formalin, small dose. | |
| | Titre of sera after vaccination and before infection : | All negative at 1 : 10. |
| | No. of guinea-pigs examined, 8. | |
| | „ „ found pregnant, 0. | |
| | „ „ infected in the uterus, 6. | |
| Group 5. | Virulent strain sterilised by formalin. | |
| | Titres of sera after vaccination and before infection : | Negative at 1 : 10–1 : 40. |
| | No. of guinea-pigs examined, 8. | |
| | „ „ found pregnant, 0. | |
| | „ „ infected in the uterus, 5. | |
| Group 6. | Controls. | |
| | No. of guinea-pigs examined, 8. | |
| | „ „ found pregnant, 0. | |
| | „ „ infected in the uterus, 4. | |

The control guinea-pigs, Group 6, were all heavily infected in the spleen and in all cases *Br. abortus* was recovered from the liver. None of the animals was pregnant at the time of the examination and in four cases macroscopic examination of the uterus showed no evidence of the animals having been pregnant. From the appearance of the uteri of the four remaining animals it is probable that they had aborted. *Br. abortus* was isolated in culture from the uteri of four of the eight guinea-pigs and the uteri of two of these four animals showed no macroscopic evidence of abortion having occurred. It is, however, probable that these two latter animals had aborted as *Br. abortus* is rarely recovered, by the technique used, from the uteri of infected non-pregnant guinea-pigs unless it be soon after abortion or from uteri showing

abscessation. The combined macroscopic and bacteriological evidence therefore indicates that six of the guinea-pigs had had infected uteri and probably had aborted.

In Group 1, vaccinated with a moderately large number of living micro-organisms of Strain 45, well-developed fœtuses were found in six of the guinea-pigs. With one of the two remaining animals it was very doubtful whether abortion had occurred, but in the other there was no evidence that the animal had been pregnant. In no case was *Br. abortus* isolated from the uterus or the placentæ, showing that the animals possessed a relatively high degree of resistance to a uterine infection. It has been found that *Br. abortus* is recoverable with much greater regularity from the placentæ of infected guinea-pigs than from the uterine mucosa of non-pregnant animals, which reflects more forcefully the immunity of the uterus in the six vaccinated pregnant animals. As a group, and as individuals, the infection of the spleen was much lighter than in the controls; indeed, two of the eight vaccinated animals were found free from infection altogether and in no instance was *Br. abortus* isolated from the liver tissue.

It is difficult to decide whether the resistance of the animals in Group 2 had been increased. Five of the animals were not pregnant and in three of these there was no macroscopic evidence of the uterus having been gravid, and *Br. abortus* was not recovered from the uteri; probably both of the others had aborted and *Br. abortus* was isolated from the uterus of one of them. The sixth guinea-pig was pregnant but its uterus was not infected. The spleens and livers showed a degree of infection comparable to that shown by the controls. The guinea-pigs do not compare favourably with those in Group 1, indicating that a single small inoculation of vaccine was relatively unsatisfactory.

In the remaining groups 3, 4 and 5 there was evidence that the majority of the animals had aborted and there was no evidence of any increased resistance to infection. It may therefore be concluded that the formalinised vaccines whether prepared from Strain 45 or from the virulent Strain 253, and this strain was used to test immunity, were of little value. Even when the formalinised vaccines were inoculated in very large quantity they were definitely very inferior to the vaccine composed of living non-virulent micro-organisms.

In conformity with what has been the general experience throughout all the experiments, the agglutination titres at the time the guinea-pigs were killed were highest among the control animals and those groups which showed no increased resistance to infection.

Attention should be directed to the pre-infection agglutination titres of the sera from some of the groups. The inoculation of very large numbers of killed micro-organisms of the virulent strain in no case produced a titre higher than 1 : 40. The inoculation of

similar numbers of killed micro-organisms of the non-virulent Strain 45 produced very similar titres, 1 : 40 again being the highest recorded. The inoculation of a relatively small number, 30 million, sterilised organisms of Strain 45 failed to produce agglutinins at all; however, 30 million of the live organisms of this strain caused the appearance of agglutinins in the majority of the animals, a titre of 1 : 160 being reached in one instance. Those animals in Group 1 inoculated with the largest number of living non-virulent organisms produced titres distinctly higher than the animals receiving five times as many killed organisms of the same strain or of the virulent strain. In all of the groups except No. 1 the titres showed a very marked rise after exposure to a small number of virulent micro-organisms. A general consideration of these results suggests : (1) That following sterilisation by formalin, Strain 45 stimulated the production of agglutinins as effectively as did the sterilised virulent strain; (2) that when the living organisms were inoculated the higher titres produced by the virulent strain depended upon the invasiveness of the strain and its multiplication and persistence for some considerable time in the tissues; (3) and that the relatively feeble production of agglutinins following the inoculation of living non-virulent micro-organisms was due to their inability to persist in the body tissues.

1935—*Experiments on Male Guinea-pigs.*

An experiment was carried out in which groups of guinea-pigs were vaccinated at different times and all infected at the same time. The chief object of the experiment was to test the length of time immunity lasted. Unfortunately no satisfactory immunity was demonstrated in any of the vaccinated groups although the methods of vaccination and of infecting the animals were regarded as comparable to those used in other experiments where a definitely increased resistance had been shown. By this time four years had elapsed since the earlier experiments had indicated that the inoculation of guinea-pigs with living organisms of Strain 45 stimulated an increased resistance to a subsequent *Br. abortus* infection. The poor results obtained in the last experiment suggested the possibility of a decided antigenic deterioration of the vaccine strain. Accordingly, arrangements were now made to compare the immunity of guinea-pigs inoculated with vaccine prepared from the ordinary sub-culture of Strain 45, with the immunity of guinea-pigs inoculated with vaccine prepared from a culture which had been passaged intermittently through a short series of guinea-pigs. In anticipation of an experiment of this nature the intermittent passage of Strain 45 had commenced in February 1935, and in June 1935 a sub-culture was available from Strain 45 which had been passaged through a series of six guinea-pigs and vaccine was prepared from it; the vaccine itself was obtained from the third sub-culture on liver agar.

For further comparative purposes a group of guinea-pigs was inoculated with a culture of Strain 45 which had been sub-cultivated 550 times since September 1931. Another group of guinea-pigs was inoculated with formalinised vaccine. Particulars of the vaccination of the groups of guinea-pigs and the test inoculation of virulent culture are as follows:—

*Group* 1.—Vaccinated with 6,148 million live organisms, Strain 45, Sub-culture X21.

*Group* 2.—Vaccinated with 6,148 million live organisms, Strain 45 passaged through six guinea-pigs, Sub-culture 3. The culture from which the vaccine was prepared consisted of rather a sparse growth, the media apparently not being up to the usual standard of quality.

*Group* 3.—The vaccine was in all respects comparable to that used for Group 2, except that the vaccine was prepared from a culture of very good growth.

*Group* 4.—Vaccinated with 6,148 million live organisms of Strain 45, Sub-culture X550.

*Group* 5.—Vaccinated with 6,148 million organisms of Strain 45, Sub-culture X21, sterilised by the action of 0·25 per cent. formalin acting at 37° C. for 48 hours.

*Group* 6.—Controls.

A test inoculation of 300 organisms was given 35 days after the inoculation of the single dose of vaccine. The strain was the same as that used in the previous experiment but the numbers of organisms used was 100 times fewer. The reduction was made because of the possibility that the test inoculation in the preceding experiment had been too severe.

The guinea-pigs were examined in equal numbers from each group at from 38 to 47 days after receiving the test inoculation. The results of the experiment are summarised in Table VII.

Accepting the absence of infection from all the organs examined as the most reliable criterion for assessing the resistance of the groups, it is evident that both Group 2 and 3, which had been vaccinated with the animal-passaged strain, were the most resistant, a comparison of these two groups with the control group showing a difference of 80 per cent. in favour of the former. The two groups 1 and 4 inoculated with vaccine prepared from Sub-culture X21 and Sub-culture X550 respectively showed a 50 per cent. greater resistance to infection than the controls, which probably reflects a definite degree of immunity produced by the vaccination. Group 5 inoculated with formalinised vaccine showed a superiority of only 20 per cent. over the controls.

Infection of the liver and kidney not being detected regularly among the controls, furnish records of limited value, as do the spleen weights. But upon whichever of these data the groups be compared, Nos. 2 and 3 are found to be the most resistant. An intermediate position is occupied by Groups 4 and 5, except that

TABLE VII.

| *Group.* | *No. of G. Pigs Examined.* | *No. found Infected.* | *No Heavily Infected in Spleen.* | *No. Infected in Liver.* | *No. Infected in Kidney.* | *Average Weight of Spleens.* | *Remarks.* |
|---|---|---|---|---|---|---|---|
| 1. Vaccinated. Str. 45, Sub. X21 | 10 | 4 | 1 | 2 | 1 | 1·32 grammes. | Two showed macroscopic lesions of the spleen. |
| 2. Vaccinated. Passaged Str. 45 | 10 | 1 | 1 | 0 | 0 | 1·13 grammes. | None showed lesions. |
| 3. Vaccinated. Passaged Str. 45 | 10 | 1 | 0 | 0 | 0 | 1·00 gramme. | None showed lesions. |
| 4. Vaccinated. Str. 45, Sub. X550 | 10 | 4 | 1 | 0 | 1 | 1·43 grammes. | Two showed macroscopic lesions of the spleen. |
| 5. Vaccinated. Str. 45, Sub. X21 Formalinised | 10 | 7 | 3 | 3 | 0 | 2·39 grammes. | Four showed macroscopic lesions of the spleen. |
| 6. Controls | 10 | 9 | 4 | 1 | 2 | 1·95 grammes. | Seven showed macroscopic lesions of the spleen and three showed macroscopic lesions of one or both epididymi. |

Even after animal passage the " ærobic " vaccine strain was not recovered from the guinea-pigs in Groups 2 and 3.

in the case of Group 5 the spleen weights being heavier than those shown by the controls suggests that in this particular respect Group 5 were no more resistant than the control animals and this therefore relegates Group 5 to a position subordinate to both Groups 1 and 4; hence the relative position of the three groups is again found to be the same as when resistance was assessed on the numbers of animals in each group found to be free from infection. The superior resistance of animals in Groups 2 and 3 suggests that animal passage had improved Strain 45 as a vaccine, without resuscitating virulence for these animals.

The test inoculation in this experiment was sufficiently severe to cause infection in nine out of ten control animals, but not too drastic to break through the resistance of all members in the vaccinated groups.

## Summary.

The immunisation of guinea-pigs inoculated with living micro-organisms of a non-virulent strain of *Br. abortus* is recorded. The strain though smooth and fully agglutinable does not stimulate the production of agglutinins in high titre even when inoculated in very large amounts. This is attributed to the lack of invasiveness and the inability of the organisms to persist in the body of the inoculated animal and not to defective or deficient antigenic structure.

Female guinea-pigs when pregnant were found highly susceptible to a uterine infection with *Br. abortus,* death of the fœtus or fœtuses and abortion frequently occurring. After abortion, abscessation of the uterus was sometimes encountered and it is regarded as specific evidence of a uterine infection with *Br. abortus*. Female guinea-pigs vaccinated with the non-virulent strain before pregnancy and infected whilst pregnant or when running with male animals, though infected in the spleen, were shown to possess a high degree of resistance to a uterine infection which was not enjoyed by control animals. Male guinea-pigs inoculated with either single or multiple large doses of the non-virulent living organisms showed in some experiments a markedly increased resistance to infection, enabling the results of the experiments to be judged by a comparison of the number of infected vaccinated animals with the number of infected control animals, to the decided advantage of the former. Evidence was obtained suggesting that vaccinated animals, though not in all cases completely resistant to an infection, showed very considerable resistance to a second or superimposed infection. This does not, however, indicate that immunity necessarily depends upon the persistence of infection. Guinea-pigs inoculated with the non-virulent strain were found to be resistant after a period of 19 weeks, the longest period tested. As there is evidence to show that the non-virulent strain rarely persists in the body of an inoculated

guinea-pig for more than three weeks, an immunity lasting for 19 weeks may be regarded as one which is independent of the persistence of infection in the body.

Comparative tests of vaccine prepared from different sub-cultures of the non-pathogenic strain, which were separated by over 200 sub-cultivations in one instance and by more than 500 in the other, do not indicate any definite antigenic deterioration caused by repeated sub-cultivation alone.

The non-virulent strain after " intermittent " passage through a series of six guinea-pigs was still non-pathogenic, but there was some evidence that as a vaccine it was superior to the non-passaged strain. The evidence is too limited to warrant any conclusion, but it indicates one direction in which further experiments should be directed.

Although in the majority of experiments clear-cut results were obtained this was not always the case. Why in a few instances vaccinated groups of guinea-pigs showed little or no more resistance than the control animals is still obscure.

The killed vaccines tested were unsatisfactory.

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# THE SERUM PROTEIN CHANGES INDUCED BY RINDERPEST VIRUS.

By M. H. French, M.A.

## Introduction.

When an animal contracts a disease resulting from parasitic infection of its tissues, antibodies appear in the blood serum which are capable, in the majority of cases, of reaction *in vitro* with the parasitic organism or with products elaborated by or from the parasite. Antibody formation results from the injection of materials of very different natures but so far the site of the antibody formation is not known, although it appears to be connected, in part, with the reticulo-endothelial system.

Although antigens may vary very greatly in form and chemical composition, it seems that the antibodies which appear in the serum are always proteins; the evidence for this has been well reviewed by Marrack.[1] Much work has been done in connection with the composition of antibodies and it has been proved by many workers that antibodies are closely bound up with the globulin fraction of the antisera, and can be precipitated from the antisera by the common salting-out methods which will remove globulin from normal serum.

The above general remarks apply to viruses as a whole. It is not known how viruses produce their toxic effects, but it has been proved on many occasions that antibodies appear in the blood stream after infection has been established.[2, 3, 4, 5, 6, 7, 8, 9, 10, 11] Further, all experimenters agree that increase of antiviral body is associated with an increase of the globulin fraction of the serum.

The amount of work done on the chemical and serological changes in the blood of cattle suffering from rinderpest is very much less than that in other branches of rinderpest research. This is largely due to the facts that rinderpest virus cannot be cultivated in media, and that the potency of anti-rinderpest sera cannot be so easily titrated as is the case when dealing with other types of antibodies (antitoxins, precipitins, agglutinins, etc.). Experimental infection of animals is therefore never set up by the injection of pure virus, but a varying amount of blood or other tissue extracts are of necessity injected. It is conceivable that these products of themselves may produce antibodies, but it has been shown that the serum proteins of cattle are not appreciably affected when normal blood, equal in amount to that injected with the virus, is given under the skin. Also the only method available for assessing the potency of an antiserum is by balancing the antiserum against the virus itself in an animal, with death or survival as the end points.

Hartley[3] first studied the serum protein changes during rinderpest infections and during immunisation and hyper-

immunisation of cattle against this virus. He showed that antibodies were produced which were invariably connected with the serum globulin. Further, his results indicated that the antibody was removed with those globulins which were precipitated on dialysis or by saturation with sodium chloride, *i.e.*, the immune body was not associated with the pseudoglobulin fraction as had been found, up to that date, for so many antitoxic sera.

Hartley's figures showed that as a result of immunisation or hyperimmunisation against this virus, the total protein and total globulin of the serum increased, whilst the albumin was diminished. Further, this increase of globulin was due chiefly to an increase of that fraction insoluble in saturated salt solution, though a small and inconstant increase was found in those globulins soluble in saturated sodium chloride.

Hornby,[4] using the method employed by Hartley, confirmed Hartley's findings on the protein changes during hyperimmunisation. During the last 15 years, however, the methods of serum protein analysis and fractionation have undergone a tremendous advance. Investigations[7, 8, 9, 10] on other viruses with these improved methods have shown that the antiviral body is not confined to one globulin but may appear in both euglobulin and pseudoglobulin fractions or be located on the border-line of the salt precipitation limits of these two fractions.[10]

Sen and Roy,[11] working in Hartley's old laboratory, repeated his work on the protein changes during an attack of rinderpest, but used modern methods. Working with six cattle they found considerable variations in the globulin fractions, and though their results confirm Hartley's finding that the greatest change is an increase of the euglobulin fraction, they also got increases of pseudoglobulin as well.

Haddow, Sen and Roy[12] isolated the euglobulin fraction of rinderpest antiserum by precipitation with one-third saturation with ammonium sulphate and compared its protective action with that of the original antiserum, and with the water insoluble precipitate obtained by Felton's technique.[13, 14] They reported upon but a few animals, and found that the potency of the water insoluble fraction was superior to that of the euglobulin fraction and that the protective value of the original serum was found to be greater than either fraction though the difference in potency of the total globulin and the water insoluble globulin was small.

In 1931, work upon the changes in the serum protein fractions during rinderpest immunisation was started at Mpwapwa, and in this article some of the results obtained are presented.

## Experimental Procedure.

Infection was set up by the subcutaneous injection of 10 c.c. citrated virulent blood obtained from an animal killed at the height of its thermal reaction to the disease. In those experiments in

which it was desired to control the severity of the reaction, 40 c.c. of anti-rinderpest serum were inoculated simultaneously and subcutaneously into the opposite side of the beast. Blood was drawn for analysis before inoculation and then at regular intervals during the course of the disease. The blood samples were allowed to clot and the serum freed from red cells and clot by centrifugation. The serum proteins were fractionated with sodium sulphate according to the method of Howe[15] so that the euglobulin referred to later represents that fraction precipitated by 14 per cent. sodium sulphate in a dilution of 1 in 29. Total globulin was precipitated by 22 per cent. sodium sulphate also in a final dilution of 1 in 29, and pseudoglobulin represents the difference between these two. The nitrogen estimations were carried out by means of a micro-Kjeldahl method.

## Part 1.—Serum Protein Changes as the Result of Fatal Infection with Rinderpest Virus.

*Ox* 3078.—This susceptible ox was bled on 18.11.31 and then inoculated with 10 c.c. of citrated virulent blood. Four days later the temperature had risen very definitely and by the seventh day his nose and eyes were running. On the fourteenth day blood-stained diarrhoea was very marked and the animal was straining very frequently. The appetite was low and decreased still further till the seventeenth day, when the ox was too weak to stand. *T. theileri* developed on the seventeenth day. Since the animal was so weak and ill, he was slaughtered 19 days after infection as being unlikely to recover. The details of his serum protein changes are given in Table I.

TABLE I.

Serum Protein changes during a fatal case of Rinderpest. (Ox 3078.)

| | 18.11.31. | 22.11.31. | 25.11.31. | 2.12.31. | 5.12.31. |
|---|---|---|---|---|---|
| Total N per cent. ... ... | 1·0423 | 1·0717 | 0·9227 | 0·7231 | 0·7574 |
| Protein N per cent. ... | 1·0059 | 1·0423 | 0·8946 | 0·6767 | 0·7238 |
| Non-Protein N per cent. ... | 0·0364 | 0·0294 | 0·0281 | 0·0464 | 0·0336 |
| Euglobulin N per cent. ... | 0·1523 | 0·2755 | 0·1403 | 0·0850 | 0·0787 |
| Total Globulin N per cent. | 0·2352 | 0·3931 | 0·2066 | 0·1555 | 0·1725 |
| Pseudoglobulin N per cent. | 0·0829 | 0·1176 | 0·0663 | 0·0705 | 0·0938 |
| Albumin N per cent. ... | 0·7707 | 0·6492 | 0·6880 | 0·5212 | 0·5513 |
| Albumin as per cent. of total Protein ... ... | 76·61 | 62·28 | 76·90 | 77·02 | 76·17 |
| Pseudoglobulin as per cent. of total Protein ... ... | 8·24 | 11·28 | 7·41 | 10·42 | 12·96 |
| Euglobulin as per cent. of total Protein ... ... | 15·14 | 26·43 | 15·68 | 12·56 | 10·87 |

As observed by Hartley, and by Sen and Roy, the course of the disease was followed by a decrease of total protein and total globulin and euglobulin, whilst the pseudoglobulin remained

fairly constant. When the fractions are expressed as a percentage of the total protein, it is noticed that the albumin remains constant whilst the euglobulin decreases steadily after the temporary rise during the first few days. The pseudoglobulin increased slightly.

*Ox* 3978.—Like the previous ox, this animal was inoculated with 10 c.c. citrated virulent blood and received no protective antiserum. The temperature rose to a maximum on the fifth day and the appetite fell steadily. By the eighth day the animal was lying down, though he still continued to pick at his food. On the eleventh day the ox had got on to his feet again and was feeding better. After this point the animal again went downhill steadily and by the end of the second week his appetite was negligible, and the general appearance was one of weakness and depression. The animal eventually died during the fourteenth night and *post-mortem* examination showed well-marked rinderpest lesions in the abomasum, cæcum and colon. The course of the disease was uncomplicated by piroplasms or trypanosomes.

The serum protein changes found in this case are presented in Table II.

TABLE II.

SERUM PROTEIN CHANGES DURING A FATAL CASE OF RINDERPEST. (Ox 3978.)

| | 9.1.35. | 14.1.35. | 20.1.35. | 24.1.35. |
|---|---|---|---|---|
| Total N per cent. ... ... ... | 1·0229 | 1·1438 | 1·2292 | 1·1970 |
| Non-Protein N per cent. ... ... | 0·0323 | 0·0329 | 0·0323 | 0·0323 |
| Total Protein N per cent. ... ... | 0·9906 | 1·1109 | 1·1969 | 1·1647 |
| Euglobulin N per cent. ... ... | 0·1342 | 0·1613 | 0·1446 | 0·1333 |
| Total Globulin N per cent. ... ... | 0·5709 | 0·6297 | 0·6892 | 0·6027 |
| Pseudoglobulin N per cent. ... ... | 0·4367 | 0·4684 | 0·5446 | 0·4694 |
| Albumin N per cent. ... ... ... | 0·4197 | 0·4812 | 0·5077 | 0·5620 |
| Albumin as per cent. of total Protein ... | 42·37 | 43·32 | 42·41 | 48·25 |
| Pseudoglobulin as per cent. of total Protein ... ... ... ... | 44·08 | 42·16 | 45·50 | 40·30 |
| Euglobulin as per cent. of total Protein | 13·55 | 14·52 | 12·09 | 11·44 |

The results obtained with this ox differ from those of other workers in that an increase of total protein, albumin and globulin took place, whilst the euglobulin fraction remained constant. On the percentage figures we see that the albumin proportions increased slightly, whilst those of the pseudoglobulin and euglobulin fractions both decreased.

*Ox* 3972.—Unlike the two previous cases, an attempt was made to protect this ox by the subcutaneous inoculation of the euglobulin equivalent of 40 c.c. anti-rinderpest serum, at the time of injection of the virus.

The temperature reaction followed a normal course in this animal and the general appearance of the ox deteriorated from the sixth day. A fall of appetite with a slight diarrhoea followed, and the weakness got worse until death took place during the fifteenth

night. *Post-mortem* examination showed only slight rinderpest lesions of the abomasum, cæcum and colon. The cause of death was rinderpest in an already cachectic animal.

This ox was in very poor condition when inoculated with the virus, and was an example of the general observation that animals in good condition withstand the disease much better than poor conditioned beasts.

TABLE III.

SERUM PROTEIN CHANGES DURING A FATAL CASE OF RINDERPEST. (Ox 3972.)

| | 9.1.35. | 13.1.35. | 18.1.35. | 24.1.35. |
|---|---|---|---|---|
| Total N per cent. ... ... ... | 1·1607 | 1·2950 | 1·2306 | 1·2082 |
| Non-Protein N per cent. ... ... | 0·0323 | 0·0327 | 0·0323 | 0·0323 |
| Total Protein N per cent. ... ... | 1·1284 | 1·2623 | 1·1983 | 1·1759 |
| Euglobulin N per cent. ... ... | 0·1325 | 0·1338 | 0·1375 | 0·1302 |
| Total globulin N per cent. ... ... | 0·5596 | 0·6009 | 0·5556 | 0·5007 |
| Pseudoglobulin N per cent. ... ... | 0·4271 | 0·4671 | 0·4181 | 0·3705 |
| Albumin N per cent. ... ... ... | 0·5688 | 0·6614 | 0·6430 | 0·6752 |
| Albumin as per cent. of total Protein... | 50·40 | 52·40 | 53·65 | 57·42 |
| Pseudoglobulin as per cent. of total Protein ... ... ... ... | 37·85 | 37·00 | 34·88 | 31·51 |
| Euglobulin as per cent. of total Protein | 11·75 | 10·60 | 11·47 | 11·07 |

The changes in this ox are again different from the above two cases. As would be expected from the results of the Muktesar workers, the total protein and globulin show an initial rise. These figures differ, however, in that the euglobulin fraction remains constant, whilst the pseudoglobulin decreases.

*Summary of Fatal Cases.*—Of the three fatal cases examined, one ox differed from those examined by previous workers in that he showed an increase of total globulin and total protein instead of the usual decrease as the disease runs its course. Like the results of Sen and Roy,[11] there is much more variation in the proportions of the different protein fractions than was found by Hartley.[3] Ox 3079 agrees with Hartley's results, *i.e.*, a decrease of euglobulin and a slight rise of pseudoglobulin, whilst Ox 3978 showed a decrease of the percentage of pseudoglobulin as well as of euglobulin, and in Ox 3972 the proportion of the euglobulin fraction remained constant but that of pseudoglobulin fell.

## PART 2.—SERUM PROTEIN CHANGES IN OXEN MAKING SPONTANEOUS RECOVERIES FOLLOWING INFECTION WITH RINDERPEST VIRUS.

*Ox* 3935.—This ox did not receive antiserum but yet made a successful recovery from rinderpest set up by the inoculation of 10 c.c. virulent blood. The temperature reaction was somewhat delayed in this case and reached its maximum on the eighth day.

Marked clinical symptoms of rinderpest were obtained in this case. The animal was off its feed on the ninth day and was very ill on the tenth day, with frequent diarrhœa and profuse nasal and lachrymal discharges. The ox first began to improve on the fourteenth day and then steadily got better again.

This case may therefore be described as one with a marked thermal and severe clinical reactions. The serum protein changes are shown in Table IV.

TABLE IV.

SERUM PROTEIN CHANGES DURING SEVERE RINDERPEST. (Ox 3935.)

| | 21.11.34. | 25.11.34. | 29.11.34. | 3.12.34. | 8.12.34. | 11.12.34. |
|---|---|---|---|---|---|---|
| Total N per cent. ... | 1·1747 | 1·4347 | 1·0101 | 0·9348 | 1·0630 | 1·0102 |
| Non-Protein N per cent. | 0·0323 | 0·0488 | 0·0318 | 0·0318 | 0·0476 | 0·0317 |
| Total Protein N per cent. ... ... ... | 1·1424 | 1·3859 | 0·9783 | 0·9030 | 1·0154 | 0·9785 |
| Euglobulin N per cent. | 0·1162 | 0·1650 | 0·2070 | 0·1822 | 0·1739 | 0·1208 |
| Total Globulin N per cent. ... ... ... | 0·5380 | 0·6391 | 0·5948 | 0·5318 | 0·5600 | 0·5072 |
| Pseudoglobulin N per cent. ... ... ... | 4·4118 | 0·4741 | 0·3878 | 0·3496 | 0·3861 | 0·3864 |
| Albumin N per cent. ... | 0·6044 | 0·7468 | 0·3835 | 0·3712 | 0·4554 | 0·4713 |
| N per cent. of fraction insoluble in saturated NaCl... ... ... | 0·1225 | 0·1911 | 0·2051 | 0·1659 | 0·2034 | 0·1834 |
| Albumin as per cent. of total Protein... ... | 52·90 | 53·89 | 39·20 | 41·11 | 44·85 | 48·16 |
| Pseudoglobulin as per cent. of total Protein | 36·04 | 34·21 | 39·64 | 38·71 | 38·02 | 39·49 |
| Euglobulin as per cent. of total Protein ... | 10·17 | 11·90 | 21·16 | 20·18 | 17·12 | 12·35 |
| Saturated NaCl insoluble Protein as per cent of total Protein... | 10·72 | 13·76 | 20·97 | 29·44 | 20·03 | 18·75 |

The main points about these figures are that the globulin fraction insoluble in saturated NaCl (as studied by Hartley[3]) is greater in amount than the euglobulin fraction estimated by Howe's method. Further, the changes in these two fractions do not run absolutely parallel though they show a similar fluctuation tendency. The proportional amount of euglobulin rises to a maximum by the eighth day and then falls slowly. The protein insoluble in saturated NaCl reaches its maximum on the twelfth day and then falls slowly. The proportional amount of pseudoglobulin falls in the first few days and then rises to a level higher than the initial value and remains constant.

*Ox* 3079.—This animal ran a typical fever after infection with rinderpest virus and by the eighth day had lost much of his normal appetite. Diarrhœa and discharges from the eyes and nose were also noticeable at this point. The animal remained very ill until the twelfth day and then began to recover. The clinical and

thermal reactions show that this animal, like Ox 3935, passed through a severe attack of rinderpest.

The serum protein changes are given in Table V.

TABLE V.

SERUM PROTEIN CHANGES DURING SEVERE RINDERPEST. (Ox 3079.)

| | 18.11.31. | 22.11.31. | 25.11.31. | 2.12.31. | 5.12.31. |
|---|---|---|---|---|---|
| Total N per cent. ... ... | 1·0899 | 1·3114 | 1·1639 | 0·9951 | 1·0026 |
| Non-Protein N per cent. ... | 0·0323 | 0·0323 | 0·0318 | 0·0318 | 0·0318 |
| Total Protein N per cent. ... | 1·0576 | 1·2791 | 1·1321 | 0·9633 | 0·9708 |
| Euglobulin N per cent. ... | 0·1177 | 0·1950 | 0·1894 | 0·1425 | 0·1500 |
| Total Globulin N per cent. | 0·5296 | 0·6391 | 0·5728 | 0·5040 | 0·5653 |
| Pseudoglobulin N per cent. | 0·4119 | 0·4441 | 0·3834 | 0·3615 | 0·4156 |
| Albumin N per cent. ... | 0·5280 | 0·6400 | 0·5593 | 0·4593 | 0·4055 |
| N per cent. of fraction insoluble in saturated NaCl | 0·1841 | 0·2911 | 0·2497 | 0·2506 | 0·1630 |
| Albumin as per cent. of Protein ... ... ... | 49·95 | 50·04 | 49·40 | 47·69 | 42·80 |
| Pseudoglobulin as per cent. of Protein ... ... | 38·90 | 34·72 | 33·87 | 37·53 | 41·76 |
| Euglobulin as per cent. of Protein ... ... ... | 11·14 | 15·24 | 16·73 | 14·79 | 15·44 |
| Saturated NaCl insoluble Protein as per cent. of total Protein ... ... | 17·41 | 22·76 | 22·06 | 26·02 | 16·79 |

The changes in this animal resemble those for the previous ox. The early rise in the total globulin and total protein was followed by a fall until the twelfth day, and then by a return to normal. The proportional changes in the euglobulin are small and less than those for the globulin insoluble in saturated NaCl. The pseudoglobulin proportion fell at first and then rose again as crisis was passed.

*Ox* 3980.—This animal was given the usual 10 c.c. of virulent blood but no antiserum. A well-marked thermal reaction followed, but no marked loss of appetite resulted. Diarrhœa and nasal discharges were absent. This ox never looked really ill and at the end of three weeks had not lost condition or weight. He was discharged after a mild rinderpest reaction which involved no clinical symptoms.

The serum protein changes are given in Table VI (page 125).

The normal fall of total protein, globulin and albumin took place with a rise in the euglobulin fraction, but a reduction of the pseudoglobulin content. When the proportional distribution of the fractions is considered it is seen that the euglobulin fraction rose steadily till the fifteenth day, whilst the pseudoglobulin and albumin both decreased. After the fifteenth day these changes moved in the opposite directions.

*Cow, Dodoma.*—This half-grade Ayrshire cow was given virulent blood without a protective antiserum and developed a

TABLE VI.

SERUM PROTEIN CHANGES DURING A MILD ATTACK OF RINDERPEST. (Ox 3980.)

| | 16.1.35. | 20.1.35. | 25.1.35. | 31.1.35. | 6.2.35. |
|---|---|---|---|---|---|
| Total N per cent. ... ... | 1·1438 | 1·1228 | 1·1198 | 1·1130 | 1·0780 |
| Non-Protein N per cent. ... | 0·0323 | 0·0323 | 0·0323 | 0·0382 | 0·0343 |
| Total Protein N per cent. ... | 1·1115 | 1·0905 | 1·0875 | 1·0748 | 1·0437 |
| Euglobulin N per cent. ... | 0·1126 | 0·1525 | 0·2109 | 0·2163 | 0·1848 |
| Total Globulin N per cent. | 0·6094 | 0·6392 | 0·6508 | 0·6480 | 0·6070 |
| Pseudoglobulin N per cent. | 0·4968 | 0·4867 | 0·4399 | 0·4317 | 0·4222 |
| Albumin N per cent. ... | 0·5021 | 0·4513 | 0·4367 | 0·4268 | 0·4367 |
| Albumin as per cent. of Protein ... ... ... | 45·17 | 41·38 | 40·15 | 39·71 | 41·84 |
| Pseudoglobulin as per cent. of Protein ... ... | 44·70 | 44·63 | 40·45 | 40·17 | 40·45 |
| Euglobulin as per cent. of Protein ... ... ... | 10·13 | 13·99 | 19·40 | 20·12 | 17·71 |

high thermal reaction by the fifth day. The appetite fell and by the seventh day the cow consumed only a little cottonseed and no roughage at all. Nasal and lachrymal discharges were absent, but diarrhœa was frequent and very pronounced. The animal began to eat again by the tenth day and diarrhœa ceased, and the cow then started to recover.

This case may be regarded as a moderately severe attack of rinderpest, with a very pronounced thermal and some clinical symptoms. The impression formed during this case was that the cottonseed supplement had swung the balance towards recovery, by tempting the appetite with a palatable and easily-digested food, whereas the cow would normally have refused to eat.

Table VII summarises the serum protein changes in this cow.

TABLE VII.

SERUM PROTEIN CHANGES DURING MODERATELY SEVERE RINDERPEST. (COW, DODOMA.)

| | 24.12.35. | 30.12.35. | 4.1.36. | 11.1.36. | 16.1.36. |
|---|---|---|---|---|---|
| Total N per cent. ... ... | 1·0640 | 1·0850 | 1·0570 | 0·8848 | 1·0318 |
| Non-Protein N per cent. ... | 0·0441 | 0·0441 | 0·0441 | 0·0343 | 0·0418 |
| Total Protein N per cent. | 1·0199 | 1·0409 | 1·0129 | 0·8505 | 0·9900 |
| Euglobulin N per cent. ... | 0·0896 | 0·0766 | 0·0826 | 0·0890 | 0·1752 |
| Total Globulin N per cent. | 0·4875 | 0·4476 | 0·4196 | 0·3854 | 0·5279 |
| Pseudoglobulin N per cent. | 0·3979 | 0·3710 | 0·3370 | 0·2964 | 0·3527 |
| Albumin N per cent. ... | 0·5324 | 0·5933 | 0·5933 | 0·4651 | 0·4621 |
| Euglobulin as per cent. of Protein ... ... ... | 8·78 | 7·36 | 8·16 | 10·47 | 17·70 |
| Pseudoglobulin as per cent. of Protein ... ... | 39·01 | 35·64 | 33·27 | 34·85 | 35·62 |
| Albumin as per cent. of Protein ... ... ... | 52·20 | 57·00 | 58·57 | 54·68 | 46·68 |

These changes show that a decrease of total protein and globulin took place. The proportional changes may be summarised as a fall in the euglobulin and pseudoglobulin fractions with a subsequent increase as crisis was passed, whilst the albumin changes are in the opposite direction.

*Summary of Cases making Spontaneous Recoveries.*—The globulin insoluble in saturated NaCl is present in greater relative and absolute amounts than the euglobulin fraction separated by 14 per cent. $Na_2SO_4$. The changes in these two fractions follow the same general fluctuation tendencies.

Hartley found that in animals which recover successfully from rinderpest without the aid of an antiserum, the protein changes at first resembled those for an animal fatally infected, but that this trend of events ceased when crisis was passed. Only one of the above animals (Cow, Dodoma) agreed exactly with Hartley's findings and showed the preliminary decrease of the relative amounts of euglobulin and pseudoglobulin with an increase of the albumin proportions, followed by an increase of the first two fractions and a decrease of the albumin as recovery progressed. The other three oxen differed in the following respects. In Oxen 3079 and 3935 the initial rise of total globulin and total protein by the fourth day is followed by a decrease until the twelfth day, when a return to normal values commences. The relative changes in the various fractions are a steady increase in the euglobulin until the end of the second week and then a slower decline. This change, without a preliminary drop, was shown also by the fraction insoluble in saturated NaCl. The pseudoglobulin and albumin percentages fall until the point of maximum euglobulin rise and then return to normal. In Ox 3980 the changes in the relative amounts follow this course, although there is a steady fall of total globulin and total protein until the end of the third week. No temporary fall of euglobulin is observed in these three cases as was found by Hartley, and in this respect these animals resemble more closely animals being immunised by the simultaneous inoculation of virulent blood and anti-rinderpest serum. These cases are all reviewed in Table XXI later.

### Part 3.—Serum Protein Changes during Immunisation against Rinderpest Virus.

For the purpose of conferring a lasting immunity on an animal the method adopted here is to inject simultaneously on opposite sides of the body 10 c.c. of virulent blood and 40 c.c. antiserum. Twelve animals were immunised and are reported upon below. Nine of these animals are divided into three groups, according to the nature of the serum protein changes involved during immunisation. The other three oxen are dealt with in Part 4.

Group A.—The four animals in this group all gave protein changes similar to those recorded by Hartley.

*Ox* 3929.—This animal was protected not by serum but by the water insoluble protein from 40 c.c. of serum. A definite thermal reaction followed, with a loss of appetite, though no diarrhœa or other discharges followed. The protein changes are exactly like those recorded for three of the oxen in Part 2.

TABLE VIII.

SERUM PROTEIN CHANGES DURING IMMUNISATION. (Ox 3929.)

| | 21.11.34. | 25.11.34. | 29.11.34. | 3.12.34. | 7.12.34. | 11.12.34 |
|---|---|---|---|---|---|---|
| Total N per cent. ... | 1·1704 | 1·2723 | 1·1005 | 1·0499 | 1·0856 | 1·0457 |
| Non-Protein N per cent. | 0·0376 | 0·0488 | 0·0318 | 0·0318 | 0·0317 | 0·0317 |
| Total Protein N per cent. ... ... ... | 1·1328 | 1·2235 | 1·0687 | 1·0181 | 1·0539 | 1·0140 |
| Euglobulin N per cent. | 0·1005 | 0·1393 | 0·1602 | 0·1535 | 0·1634 | 0·1420 |
| Total Globulin N per cent. ... ... ... | 0·5932 | 0·6776 | 0·6632 | 0·8034 | 0·6128 | 0·5627 |
| Pseudoglobulin N per cent. ... ... ... | 0·4927 | 0·5383 | 0·5030 | 0·4499 | 0·4494 | 0·4207 |
| Albumin N per cent. ... | 0·5396 | 0·5459 | 0·4055 | 0·4147 | 0·4411 | 0·4513 |
| N per cent. of fraction insoluble in saturated NaCl... ... ... | 0·1382 | 0·1901 | 0·2656 | 0·2601 | 0·2411 | 0·1901 |
| Albumin as per cent. of Protein ... ... | 47·63 | 44·62 | 33·14 | 40·73 | 41·86 | 44·51 |
| Pseudoglobulin as per cent. of Protein ... | 43·50 | 44·00 | 41·11 | 44·19 | 48·64 | 41·49 |
| Euglobulin as per cent. of Protein ... ... | 8·87 | 11·38 | 15·54 | 15·08 | 15·50 | 14·00 |
| Saturated NaCl insoluble Protein as per cent. of total Protein | 12·20 | 15·54 | 21·71 | 25·55 | 22·88 | 18·75 |

TABLE IX.

SERUM PROTEIN CHANGES DURING IMMUNISATION. (Ox 3933.)

| | 21.11.34. | 26.11.34. | 30.11.34. | 3.12.34. | 11.12.34. |
|---|---|---|---|---|---|
| Total N per cent. ... ... | 1·0513 | 1·2484 | 1·1414 | 1·0704 | 1·0027 |
| Non-Protein N per cent. ... | 0·0323 | 0·0318 | 0·0318 | 0·0318 | 0·0325 |
| Protein N per cent. ... | 1·0190 | 1·2166 | 1·1096 | 1·0386 | 0·9702 |
| Euglobulin N per cent. ... | 0·1446 | 0·2516 | 0·2669 | 0·2385 | 0·1782 |
| Total Globulin N per cent. | 0·5254 | 0·6100 | 0·5730 | 0·5674 | 0·5197 |
| Pseudoglobulin N per cent. | 0·3808 | 0·3584 | 0·3061 | 0·3289 | 0·3415 |
| Albumin N per cent. ... | 0·4936 | 0·6066 | 0·5366 | 0·4712 | 0·4505 |
| N per cent. of fraction insoluble in saturated NaCl | 0·1629 | 0·2102 | 0·3272 | 0·2960 | 0·2638 |
| Albumin as per cent. of Protein ... ... ... | 48·44 | 49·86 | 48·36 | 45·37 | 46·43 |
| Pseudoglobulin as per cent. of Protein ... ... | 37·37 | 29·46 | 27·59 | 31·67 | 35·20 |
| Euglobulin as per cent. of Protein ... ... ... | 14·19 | 20·67 | 24·05 | 22·96 | 18·37 |
| Saturated NaCl insoluble fraction as per cent. of Protein ... ... ... | 15·99 | 17·26 | 29·49 | 28·50 | 27·19 |

*Ox* 3933.—This animal was protected by 40 c.c. of anti-rinderpest serum. A definite thermal reaction followed with a slight loss of appetite and condition. The changes in this ox are of the same nature as those recorded for Ox 3929.

*Ox* 3971.—This ox was protected by the euglobulin obtained from 40 c.c. of anti-rinderpest serum. Only a mild reaction followed. The same type of changes were found in the serum proteins.

TABLE X.

SERUM PROTEIN CHANGES DURING IMMUNISATION. (Ox 3971.)

| | 9.1.35. | 13.1.35. | 18.1.35. | 24.1.35. | 29.1.35. |
|---|---|---|---|---|---|
| Total N per cent. ... ... | 1·2190 | 1·2908 | 1·2306 | 0·9198 | 1·2082 |
| Non-Protein N per cent. ... | 0·0293 | 0·0327 | 0·0329 | 0·0323 | 0·0382 |
| Total Protein N per cent.... | 1·1897 | 1·2581 | 1·1977 | 0·8875 | 1·1700 |
| Euglobulin N per cent. ... | 0·1003 | 0·1478 | 0·2094 | 0·1971 | 0·2161 |
| Total globulin N per cent. | 0·6487 | 0·6658 | 0·6665 | 0·5057 | 0·6416 |
| Pseudoglobulin N per cent. | 0·5484 | 0·5180 | 0·4571 | 0·3086 | 0·4255 |
| Albumin N per cent. ... | 0·5410 | 0·5923 | 0·5312 | 0·3818 | 0·5284 |
| Albumin as per cent. of Protein ... ... ... | 47·16 | 47·08 | 44·35 | 43·02 | 45·16 |
| Pseudoglobulin as per cent. of Protein ... ... | 44·41 | 41·17 | 38·16 | 34·77 | 36·37 |
| Euglobulin as per cent. of Protein ... ... ... | 8·43 | 11·75 | 17·49 | 22·21 | 18·47 |

TABLE XI.

SERUM PROTEIN CHANGES DURING IMMUNISATION. (Ox 3932.)

| | 21.11.34. | 26.11.34. | 30.11.34. | 3.12.34. | 11.12.34. |
|---|---|---|---|---|---|
| Total N per cent. ... ... | 1·0667 | 1·1071 | 1·1157 | 1·0499 | 1·1306 |
| Non-Protein N per cent. ... | 0·0323 | 0·0421 | 0·0318 | 0·0376 | 0·0317 |
| Protein N per cent. ... | 1·0344 | 1·0650 | 1·0839 | 1·0123 | 1·0989 |
| Euglobulin N per cent. ... | 0·0882 | 0·1486 | 0·2162 | 0·2575 | 0·1469 |
| Total Globulin N per cent. | 0·5164 | 0·5160 | 0·5346 | 0·6034 | 0·5898 |
| Pseudoglobulin N per cent. | 0·4282 | 0·3674 | 0·3184 | 0·3459 | 0·4429 |
| Albumin N per cent. ... | 0·5180 | 0·5490 | 0·5493 | 0·4089 | 0·5091 |
| N per cent. of fraction insoluble in saturated NaCl | 0·1076 | 0·3004 | 0·4673 | 0·3563 | 0·2957 |
| Albumin as per cent. of Protein ... ... ... | 50·09 | 51·55 | 50·68 | 40·39 | 46·33 |
| Pseudoglobulin as per cent. of Protein ... ... | 41·40 | 34·50 | 29·37 | 34·18 | 40·30 |
| Euglobulin as per cent. of Protein ... ... ... | 8·52 | 13·95 | 19·94 | 25·44 | 13·37 |
| Saturated NaCl insoluble fraction as per cent. of Protein ... ... ... | 10·40 | 28·11 | 43·11 | 35·21 | 26·90 |

*Ox* 3932.—Protection was afforded by means of 40 c.c. of anti-rinderpest serum and only a thermal reaction was obtained. A complication by the appearance of *T. theileri* on the sixteenth

day after infection reduces the significance of the serum protein changes for this ox, but since they are of the same nature as for the previous three oxen they are presented up to the sixteenth day only.

The changes of serum proteins in these four animals may be summarised as a fall of total protein and pseudoglobulin together with a rise of total globulin and particularly of the euglobulin and the fraction insoluble in saturated NaCl. In three cases the albumin fell, whilst in the other it remained nearly constant. The relative amount of euglobulin and of protein insoluble in saturated NaCl increased steadily until the end of the second week and then fell slowly. The albumin and pseudoglobulin proportions decreased until the maximum euglobulin content was reached and then increased slowly. These changes are exactly as found by Hartley and by Sen and Roy.

Group B.—Three animals are included in this group because they behaved similarly, but in a different way from those in Group A.

*Ox* 3973.—This animal received protection by the inoculation of the water insoluble protein from 40 c.c. anti-rinderpest serum. Only a mild thermal reaction, with very slight loss of appetite and no clinical symptoms, developed in this ox.

TABLE XII.

Serum Protein changes during Immunisation. (Ox 3973.)

| | 9.1.35. | 14.1.35. | 19.1.35. | 29.1.35. |
|---|---|---|---|---|
| Total N per cent. ... ... ... | 1·1603 | 1·1284 | 1·0010 | 1·1416 |
| Non-Protein N per cent. ... ... | 0·0323 | 0·0329 | 0·0323 | 0·0382 |
| Total Protein N per cent. ... ... | 1·1280 | 1·0955 | 0·9687 | 1·1034 |
| Euglobulin N per cent. ... ... | 0·1034 | 0·1296 | 0·1821 | 0·1994 |
| Total Globulin N per cent. ... ... | 0·5858 | 0·5925 | 0·5869 | 0·6661 |
| Pseudoglobulin N per cent. ... ... | 0·4842 | 0·4629 | 0·4048 | 0·4667 |
| Albumin N per cent. ... ... ... | 0·5422 | 0·5030 | 0·3818 | 0·4373 |
| Albumin as per cent. of Protein ... | 48·07 | 45·92 | 39·41 | 39·63 |
| Pseudoglobulin as per cent. of Protein | 42·76 | 42·25 | 41·79 | 42·30 |
| Euglobulin as per cent. of Protein ... | 9·17 | 11·83 | 18·80 | 18·07 |

*Ox* 3976.—Forty c.c. of anti-rinderpest serum gave this ox protection from the virus. A thermal reaction attended by a very slight loss of appetite and no clinical symptoms marked the immunisation of this ox.

*Ox* 3931.—The euglobulin obtained from 40 c.c. of anti-rinderpest serum was used to protect this ox. A definite thermal reaction followed but no clinical symptoms appeared. On the sixteenth day the animal was found to be harbouring *T. theileri* and so the analyses were discontinued at this point. Although this animal has been included in this paper because the serum protein

changes are of the same order as for other animals undergoing rinderpest immunisation, some reservation must be attached to the significance of these figures.

TABLE XIII.

SERUM PROTEIN CHANGES DURING IMMUNISATION. (Ox 3976.)

| | 9.1.35. | 13.1.35. | 18.1.35. | 24.1.35. | 30.1.35. |
|---|---|---|---|---|---|
| Total N per cent. ... ... | 1·1029 | 1·2740 | 1·0584 | 1·0584 | 1·0276 |
| Non-Protein N per cent. ... | 0·0323 | 0·0329 | 0·0323 | 0·0323 | 0·0382 |
| Total Protein N per cent.... | 1·0706 | 1·2411 | 1·0261 | 1·0261 | 0·9894 |
| Euglobulin N per cent. ... | 0·1394 | 0·2103 | 0·1872 | 0·1950 | 0·1654 |
| Total Globulin N per cent. | 0·6018 | 0·7295 | 0·6483 | 0·6443 | 0·6054 |
| Pseudoglobulin N per cent. | 0·4624 | 0·5192 | 0·4611 | 0·4493 | 0·4400 |
| Albumin N per cent. ... | 0·4688 | 0·5116 | 0·3778 | 0·3818 | 0·3840 |
| Albumin as per cent. of Protein ... ... ... | 43·79 | 41·22 | 36·83 | 37·21 | 38·81 |
| Pseudoglobulin as per cent. of Protein ... ... | 43·19 | 41·83 | 44·93 | 43·79 | 44·47 |
| Euglobulin as per cent. of Protein ... ... ... | 13·92 | 16·95 | 18·24 | 19·00 | 16·72 |

TABLE XIV.

SERUM PROTEIN CHANGES DURING IMMUNISATION. (Ox 3931.)

| | 21.11.34. | 25.11.34. | 29.11.34. | 3.12.34. | 11.12.34. |
|---|---|---|---|---|---|
| Total N per cent. ... ... | 1·0511 | 1·4192 | 1·0025 | 1·0141 | 1·0820 |
| Non-Protein N per cent. ... | 0·0318 | 0·0488 | 0·0318 | 0·0318 | 0·0323 |
| Protein N per cent. ... | 1·0193 | 1·3704 | 0·9707 | 0·9823 | 1·0497 |
| Euglobulin N per cent. ... | 0·1319 | 0·2392 | 0·2406 | 0·2269 | 0·2075 |
| Total Globulin N per cent. | 0·5131 | 0·7469 | 0·6091 | 0·6168 | 0·5990 |
| Pseudoglobulin N per cent. | 0·3812 | 0·5077 | 0·3685 | 0·3899 | 0·3915 |
| Albumin N per cent. ... | 0·5062 | 0·6235 | 0·3616 | 0·3655 | 0·4507 |
| N per cent. of fraction insoluble in saturated NaCl | 0·1381 | 0·1816 | 0·2580 | 0·2903 | 0·2736 |
| Albumin as per cent. of Protein ... ... ... | 49·67 | 45·51 | 37·24 | 41·29 | 42·98 |
| Pseudoglobulin as per cent. of Protein ... ... | 37·40 | 37·05 | 37·97 | 35·63 | 37·34 |
| Euglobulin as per cent. of Protein ... ... ... | 12·94 | 17·45 | 24·79 | 23·10 | 19·78 |
| Saturated NaCl insoluble fraction as per cent. of Protein ... ... ... | 13·55 | 13·26 | 26·58 | 29·56 | 26·07 |

The serum protein changes during the immunisation of these three oxen differ from those found in the oxen of Group A in that, whilst the relative proportion of euglobulin increased, the proportional amount of pseudoglobulin remained constant instead of decreasing in the same way as did the albumin. There was a fall of total protein but the total globulin content increased as with the animals of Group A.

GROUP C.—The serum protein changes of the two oxen in this group differ from those of the previous groups.

*Ox* 3974.—This animal was protected with the water insoluble protein of 40 c.c. anti-rinderpest serum. A thermal reaction with no clinical symptoms and no appreciable loss of appetite characterised this immunisation.

TABLE XV.

SERUM PROTEIN CHANGES DURING IMMUNISATION. (Ox 3974.)

| | 9.1.35. | 14.1.35. | 19.1.35. | 29.1.35. |
|---|---|---|---|---|
| Total N per cent. ... ... ... | 1·0752 | 1·1374 | 1·0374 | 1·0024 |
| Non-Protein N per cent. ... ... | 0·0323 | 0·0329 | 0·0323 | 0·0328 |
| Total Protein N per cent. ... ... | 1·0429 | 1·1045 | 1·0051 | 0·9696 |
| Euglobulin N per cent.... ... ... | 0·1108 | 0·1280 | 0·1871 | 0·1756 |
| Total globulin N per cent. ... ... | 0·5549 | 0·6058 | 0·6233 | 0·6036 |
| Pseudoglobulin N per cent. ... ... | 0·4441 | 0·4778 | 0·4362 | 0·4280 |
| Albumin N per cent. ... ... ... | 0·4880 | 0·4987 | 0·3818 | 0·3660 |
| Albumin as per cent. of Protein ... | 46·80 | 45·15 | 37·99 | 37·75 |
| Pseudoglobulin as per cent. of Protein | 42·58 | 43·26 | 43·40 | 44·14 |
| Euglobulin as per cent. of Protein ... | 10·62 | 11·59 | 18·61 | 18·11 |

*Ox* 3975.—Anti-rinderpest serum was given at the same time as the virulent blood and a thermal reaction with no clinical symptoms followed.

TABLE XVI.

SERUM PROTEIN CHANGES DURING IMMUNISATION. (Ox 3975.)

| | 9.1.35. | 13.1.35. | 18.1.35. | 24.1.35. | 30.1.35. |
|---|---|---|---|---|---|
| Total N per cent. ... ... | 1·0325 | 1·0798 | 1·0164 | 1·1438 | 1·0024 |
| Non-Protein N per cent.... | 0·0323 | 0·0329 | 0·0323 | 0·0323 | 0·0382 |
| Total Protein N per cent.... | 1·0002 | 1·0469 | 0·9841 | 1·1115 | 0·9642 |
| Euglobulin N per cent. ... | 0·1363 | 0·1486 | 0·2070 | 0·2235 | 0·1564 |
| Total Globulin N per cent. | 0·5388 | 0·5910 | 0·6023 | 0·7297 | 0·6030 |
| Pseudoglobulin N per cent. | 0·4025 | 0·4424 | 0·3953 | 0·5062 | 0·4466 |
| Albumin N per cent. ... | 0·4614 | 0·4559 | 0·3818 | 0·3818 | 0·3612 |
| Albumin as per cent. of Protein ... ... ... | 46·13 | 43·55 | 38·80 | 34·35 | 37·46 |
| Pseudoglobulin as per cent. of Protein ... ... | 40·24 | 42·26 | 40·17 | 45·54 | 46·32 |
| Euglobulin as per cent. of Protein ... ... ... | 13·63 | 14·19 | 21·03 | 20·11 | 16·22 |

The serum protein changes of these two cases differ from the previous ones and from those of Hartley in that there was an increase in the relative amount of pseudoglobulin as well as an increase of the euglobulin fraction.

## Part 4.—To Determine to what Extent the Serum Protein Changes during Immunisation are Caused by the Loss of Appetite.

For this experiment six susceptible six-tooth oxen from the Kondoa-Irangi district were chosen. The animals had been grazed locally for about a month and were in moderate condition. Their weights were very comparable and nothing distinguished one ox from another except colour.

Three of these oxen were housed in separate stalls in the rinderpest hospital. On 13.10.34 they were numbered and given 10 c.c. citrated virulent blood and 30 c.c. anti-rinderpest serum simultaneously into opposite sides of the body. A dose of 30 c.c. of serum was given instead of the routine 40 c.c. so as to ensure that the animals would show definite symptoms.

These animals were allowed an *ad lib.* ration of hay. A weighed quantity of hay was fed one day and then all the hay uneaten at 7 a.m. the next day was weighed and subtracted from the total fed to measure the daily consumption.

The three remaining oxen were also housed in separate stalls in another hospital and precautions taken to prevent any possibility of infection with rinderpest virus. Each of these three controls was given a hay ration equal in quantity to that consumed in the previous day by his corresponding experimental ox. By this means it was possible to keep the controls under the exact conditions as the experimental animals.

When such oxen are brought into hospital for the first time it takes them some time to settle down to their new environment; this effect, however, would be equally operative in both groups.

TABLE XVII.

Serum Protein changes during Immunisation of Ox 3916 compared with those for his Control Ox.

Ox 3916.

| | 31.10.34. | 4.11.34. | 8.11.34. | 12.11.34. | 16.11.34. | 21.11.34. |
|---|---|---|---|---|---|---|
| Total N per cent. ... | 1·1043 | 1·1518 | 1·0369 | 1·1517 | 1·1839 | 1·1076 |
| Non-Protein N per cent. | 0·0241 | 0·0390 | 0·0241 | 0·0164 | 0·0162 | 0·0323 |
| Protein N per cent. ... | 1·0802 | 1·1128 | 1·0128 | 1·1353 | 1·1677 | 1·0753 |
| Euglobulin N per cent. | 0·0853 | 0·1202 | 0·1592 | 0·2656 | 0·2527 | 0·1991 |
| Total Globulin N per cent. ... ... ... | 0·5342 | 0·5817 | 0·5668 | 0·6584 | 0·6563 | 0·6250 |
| Pseudoglobulin N per cent. ... ... ... | 0·4489 | 0·4615 | 0·4076 | 0·3928 | 0·4036 | 0·4259 |
| Albumin N per cent. ... | 0·5460 | 0·5311 | 0·4460 | 0·4769 | 0·5114 | 0·4493 |
| Albumin as per cent. of Protein ... ... | 50·55 | 47·73 | 44·04 | 42·01 | 43·80 | 41·78 |
| Pseudoglobulin as per cent. of Protein ... | 41·55 | 41·47 | 41·24 | 34·60 | 34·56 | 39·61 |
| Euglobulin as per cent. of Protein ... ... | 7·90 | 10·80 | 15·72 | 23·39 | 21·64 | 18·51 |

Ox Mweusi.

(Control.)

| | 31.11.34. | 4.11.34. | 8.11.34. | 12.11.34. | 16.11.34. | 21.11.34. |
|---|---|---|---|---|---|---|
| Total N per cent. ... | 1·1846 | 1·1805 | 1·0976 | 1·1593 | 1·1872 | 1·1677 |
| Non-Protein N per cent. | 0·0241 | 0·0241 | 0·0323 | 0·0323 | 0·0323 | 0·0323 |
| Protein N per cent. ... | 1·1605 | 1·1664 | 1·0653 | 1·1270 | 1·1549 | 1·1354 |
| Euglobulin N per cent. | 0·1323 | 0·1389 | 0·1204 | 0·1281 | 0·1310 | 0·1256 |
| Total Globulin N per cent. ... ... ... | 0·6008 | 0·5877 | 0·5509 | 0·5990 | 0·6093 | 0·5934 |
| Pseudoglobulin N per cent. ... ... ... | 0·4685 | 0·4588 | 0·4305 | 0·4709 | 0·4783 | 0·4678 |
| Albumin N per cent. ... | 0·5597 | 0·5687 | 0·5144 | 0·5280 | 0·5456 | 0·5420 |
| Albumin as per cent. of Protein ... ... | 48·23 | 48·76 | 48·29 | 46·85 | 47·25 | 47·74 |
| Pseudoglobulin as per cent. of Protein ... | 40·37 | 39·42 | 40·41 | 41·78 | 41·41 | 41·20 |
| Euglobulin as per cent. of Protein ... ... | 11·40 | 11·91 | 11·30 | 11·37 | 11·34 | 11·06 |

There are many records in the literature of nutrition to show that complete starvation affects not only the total serum protein but also the ratio of globulin to albumin. During an attack of rinderpest there is a considerable loss of appetite and it is conceivable that this might influence the protein fractions quite apart from any effects due to the disease itself. This experiment will therefore differentiate between any serum protein changes brought about by immunisation and those resulting from a lowered food intake and reaction to confinement in a strange environment.

The serum from each control ox was drawn the day following that on which serum had been taken from the experimental ox. The results of the analyses for each pair of animals are given in Table XVII.

In spite of a reduced amount of protective serum Ox 3916 underwent a mild course of infection and showed practically no clinical symptoms but a definite and well-marked thermal reaction. No piroplasm complications disturbed the course of immunisation. The control ox Mweusi remained healthy and consumed completely the ration of hay fed to him.

Ox 3917 behaved in a similar way to the Ox 3916, and was discharged at the end of the experiment after a definite thermal reaction with practically no clinical symptoms. The control ox Mwekundu remained healthy, but on the tenth day he refused to eat his ration completely. The hay was not removed and though he did not completely eat the hay fed on subsequent days his appetite returned and his total consumption in the three weeks was less than a kilo below that of Ox 3917.

TABLE XVIII.

SERUM PROTEIN CHANGES DURING IMMUNISATION OF OX 3917 COMPARED WITH THOSE FOR HIS CONTROL OX.

OX 3917. (EXPERIMENTAL.)

| | 31.10.34. | 4.11.34. | 8.11.34. | 12.11.34. | 16.11.34. | 21.11.34. |
|---|---|---|---|---|---|---|
| Total N per cent. ... | 1·1833 | 1·1586 | 1·0944 | 1·2180 | 1·2720 | 1·2727 |
| Non-Protein N per cent. | 0·0241 | 0·0390 | 0·0241 | 0·0323 | 0·0528 | 0·0323 |
| Protein N per cent. ... | 1·1592 | 1·1196 | 1·0703 | 1·0857 | 1·0192 | 1·2404 |
| Euglobulin N per cent. | 0·0896 | 0·1644 | 0·2426 | 0·1868 | 0·1738 | 0·1936 |
| Total Globulin N per cent. ... ... ... | 0·6409 | 0·6052 | 0·6488 | 0·6350 | 0·5894 | 0·7451 |
| Pseudoglobulin N per cent. ... ... ... | 0·5513 | 0·4408 | 0·4062 | 0·4482 | 0·4156 | 0·5515 |
| Albumin N per cent. ... | 0·5183 | 0·5144 | 0·4215 | 0·4507 | 0·4298 | 0·4953 |
| Albumin as per cent. of Protein ... ... | 44·71 | 45·94 | 39·38 | 41·51 | 42·17 | 39·93 |
| Pseudoglobulin as per cent. of Protein ... | 47·56 | 39·38 | 37·95 | 41·29 | 40·78 | 44·47 |
| Euglobulin as per cent. of Protein ... ... | 7·73 | 14·68 | 22·67 | 17·20 | 17·05 | 15·60 |

OX MWEKUNDU. (CONTROL.)

| | 31.10.34. | 4.11.34. | 8.11.34. | 12.11.34. | 16.11.34. | 21.11.34. |
|---|---|---|---|---|---|---|
| Total N per cent. ... | 1·1043 | 1·1660 | 1·1517 | 1·1902 | 1·2799 | 1·0871 |
| Non-Protein N per cent. | 0·0241 | 0·0241 | 0·0323 | 0·0323 | 0·0528 | 0·0323 |
| Protein N per cent. ... | 1·0802 | 1·1419 | 1·1194 | 1·1579 | 1·2271 | 1·0548 |
| Euglobulin N per cent. | 0·1434 | 0·1550 | 0·1508 | 0·1679 | 0·1487 | 0·1299 |
| Total Globulin N per cent. ... ... ... | 0·6134 | 0·6544 | 0·6401 | 0·6572 | 0·6817 | 0·5938 |
| Pseudoglobulin N per cent. ... ... ... | 0·4700 | 0·4994 | 0·4893 | 0·4893 | 0·5330 | 0·4639 |
| Albumin N per cent. ... | 0·4668 | 0·4875 | 0·4793 | 0·5007 | 0·5454 | 0·4610 |
| Albumin as per cent. of Protein ... ... | 43·21 | 42·69 | 42·82 | 43·25 | 44·45 | 43·71 |
| Pseudoglobulin as per cent. of Protein ... | 43·51 | 43·73 | 43·71 | 42·25 | 43·43 | 43·98 |
| Euglobulin as per cent. of Protein ... ... | 13·28 | 13·58 | 13·47 | 14·50 | 12·12 | 12·31 |

Ox 3918 differed in no way from the two previous cases and was discharged after a definite thermal reaction with practically no clinical symptoms. Like the previous control ox this control, Madowa, also lost his appetite on the tenth day, but, although his appetite returned immediately, he did not make up the loss in hay consumption and at the end of the experiment had consumed 1·5 kilos of hay less than Ox 3918.

During immunisation the total protein content of the sera of Oxen 3916 and 3917 fell till the tenth day and then increased rapidly. In the case of Ox 3918 the total protein fell until the sixteenth day before it began to rise again. The absolute amounts

of total globulin do not follow a smooth curve but show a general increase in all oxen. The albumin content of the serum fell in each case to a minimum at the point of maximum globulin content. The euglobulin fraction increased, whilst that of the pseudo-globulin fell.

TABLE XIX.

SERUM PROTEIN CHANGES DURING IMMUNISATION OF OX 3918 COMPARED WITH THOSE FOR HIS CONTROL OX.

OX 3918. (EXPERIMENTAL.)

| | 31.10.34. | 4.11.34. | 8.11.34. | 12.11.34. | 16.11.34. | 21.11.34. |
|---|---|---|---|---|---|---|
| Total N per cent. ... | 1·1833 | 1·1948 | 1·1053 | 1·0326 | 1·0636 | 1·2024 |
| Non-Protein N per cent. | 0·0241 | 0·0390 | 0·0405 | 0·0323 | 0·0323 | 0·0323 |
| Protein N per cent. ... | 1·1592 | 1·1558 | 1·0648 | 1·0003 | 1·0313 | 1·1701 |
| Euglobulin N per cent. | 0·1321 | 0·1424 | 0·1591 | 0·2123 | 0·1974 | 0·1939 |
| Total Globulin N per cent. ... ... ... | 0·6409 | 0·6414 | 0·5897 | 0·5723 | 0·5763 | 0·6198 |
| Pseudoglobulin N per cent. ... ... ... | 0·5088 | 0·4990 | 0·4306 | 0·3600 | 0·3789 | 0·4259 |
| Albumin N per cent. ... | 0·5183 | 0·5144 | 0·4751 | 0·4280 | 0·4550 | 0·5503 |
| Euglobulin as per cent. of Protein ... ... | 11·39 | 12·32 | 14·94 | 21·23 | 19·14 | 16·58 |
| Pseudoglobulin as per cent. of Protein ... | 43·90 | 43·18 | 40·44 | 36·00 | 36·74 | 36·40 |
| Albumin as per cent. of Protein ... ... | 44·71 | 44·50 | 44·62 | 42·27 | 44·12 | 47·02 |

OX MADOWA. (CONTROL.)

| | 31.10.34. | 4.11.34. | 8.11.34. | 12.11.34. | 16.11.34. | 21.11.34. |
|---|---|---|---|---|---|---|
| Total N per cent. ... | 1·1833 | 1·1805 | 1·1593 | 1·1902 | 1·1640 | 1·2180 |
| Non-Protein N per cent. | 0·0150 | 0·0394 | 0·0323 | 0·0323 | 0·0323 | 0·0323 |
| Protein N per cent. ... | 1·1683 | 1·1411 | 1·1270 | 1·1579 | 1·1317 | 1·1857 |
| Euglobulin N per cent. | 0·1098 | 0·1363 | 0·1308 | 0·1390 | 0·1384 | 0·1348 |
| Total Globulin N per cent. ... ... ... | 0·6011 | 0·6271 | 0·5763 | 0·6072 | 0·5976 | 0·6443 |
| Pseudoglobulin N per cent. ... ... ... | 0·4913 | 0·4904 | 0·4455 | 0·4682 | 0·4592 | 0·4895 |
| Albumin N per cent. ... | 0·5672 | 0·5140 | 0·5507 | 0·5507 | 0·5341 | 0·5614 |
| Euglobulin as per cent. of Protein ... ... | 9·40 | 11·95 | 11·61 | 12·00 | 12·23 | 11·37 |
| Pseudoglobulin as per cent. of Protein ... | 42·05 | 43·01 | 39·53 | 40·44 | 40·57 | 41·28 |
| Albumin as per cent. of Protein ... ... | 48·55 | 45·04 | 48·86 | 47·56 | 47·20 | 47·35 |

The largest relative change is in the euglobulin fraction. There is a very marked increase until about the twelfth day, after which it begins to fall again. The pseudoglobulin fell slowly until the euglobulin reached its maximum but then increased again. The net result on the total globulin fraction was that in Oxen 3916 and 3917 there was a steady rise in the proportion of total globulin, because the relative amount of albumin decreased and the

euglobulin increase was greater than the decrease of pseudo-globulin. On the other hand, in Ox 3918 the albumin and total globulin proportions remained unchanged and the euglobulin increase appears to have taken place at the expense of pseudo-globulin.

When we turn to the analyses of the serum from the control animals we see at once a very significant difference. For the controls there was no significant alteration in the absolute and relative quantities of the various fractions.

This means that neither the loss of appetite nor the nervous excitement following removal to new surroundings have altered the serum protein content. The changes in the three experimental oxen can therefore be legitimately ascribed to the effect of the virus.

The hay consumption (in kilos), together with the live weight changes (lbs.) during the three weeks' immunisation period are given in Table XX, and compared with those for the control animals. The daily hay consumptions are not given, but the amount eaten fell, after about the fourth day, to its minimum about the eighth or ninth day and then gradually improved.

TABLE XX.

Live Weights and Food Consumption.

| | *Hay Eaten.* (*kilos.*) | *Initial Weight.* (*lb.*) | *Final Weight.* (*lb.*) | *Gain in Live Weight.* (*lb.*) |
|---|---|---|---|---|
| Ox 3916... ... ... | 52·10 | 408 | 350 | —58 |
| Ox 3917... ... ... | 55·23 | 448 | 398 | —50 |
| Ox 3918... ... ... | 62·20 | 474 | 420 | —54 |
| Ox Mweusi ... ... | 52·10 | 445 | 445 | ± 0 |
| Ox Mwekundu... ... | 54·80 | 433 | 404 | —29 |
| Ox Madowa ... ... | 60·71 | 441 | 441 | ± 0 |

As the food consumed during the experiment was the same for both groups, and since the slight diarrhœa in the immunised oxen did not persist there would be no difference in the change of the stomach contents between the groups during the period of the experiment and so loss of weight from this cause can be disregarded. The virus infection with its resulting pyrexia is therefore responsible for the loss of weight, because only one control ox lost weight and then only two-fifths of that lost by his corresponding experimental ox.

## Review of Cases Investigated.

Before proceeding to a general discussion it is perhaps worth while to review the cases examined in this investigation. This has been done in Table XXI.

TABLE XXI.

REVIEW OF CASES INVESTIGATED.

| *Treatment.* | *No. of Cattle.* | *Result.* | *Serum Globulin Changes.* | | |
|---|---|---|---|---|---|
| | | | *No. of Cases.* | *Euglobulin.* | *Pseudoglobulin.* |
| Virulent blood only | 6 | 2 died | 1 | Decreased | Decreased. |
| | | | 1 | Decreased | Remained constant. |
| | | 4 survived | 3 | Increased | Temporary decrease. |
| | | | 1 | Temporary decrease followed by increase | Temporary decrease. |
| Virulent blood and anti-serum | 13 | 1 died | 1 | Constant | Decreased. |
| | | 12 survived | 7 | Increased | Temporary decrease. |
| | | | 3 | „ | Constant. |
| | | | 2 | „ | Increased. |

## DISCUSSION.

The work of previous workers at the Muktesar laboratory has been confirmed in that definite variations have been found in the serum proteins of an ox during either a fatal attack of rinderpest or during immunisation against this virus. Hartley's original observation, that the changes during immunisation differ from those during a fatal attack of rinderpest, has been confirmed. The analyses of the three control animals in Part 4 support Hartley's conclusion that repeated small bleedings do not affect the composition of the serum. It has also been shown that in the mild reactions encountered during immunisation there is no significant alteration of the serum proteins due to the slight loss of appetite. The marked changes in the serum proteins can therefore be attributed to the effect of the virus infection.

In those cases in which estimations of the protein insoluble in saturated NaCl have been made, the observations of Hartley have been confirmed that a large increase of the relative amount of this fraction was a very marked characteristic of immunisation.

For the majority of estimations, methods evolved since the time of Hartley's work have been employed. An attempt has been made to separate the serum globulin into portions closer to the classic euglobulin and pseudoglobulin than was done by Hartley. As was found by Sen and Roy,[11] the variations of these fractions are less constant than the variation of the proteins soluble or insoluble in saturated NaCl.

The euglobulin estimated in this work tends to follow the same general changes as the protein insoluble in saturated NaCl,

although the euglobulin represents a smaller relative and absolute amount of the total globulin.

In fatally infected animals, Hartley observed a steady fall of the proteins insoluble in saturated salt. In this series, three oxen died of rinderpest and the results suggest that either a fall of euglobulin or pseudoglobulin, or of both fractions may take place as the disease gradually kills a beast.

Hartley showed that in animals which recovered from rinderpest infection without the aid of antiserum, the serum protein changes at first resembled those in an animal fatally infected, but that, as crisis passed, a steady rise took place in the proportion of protein insoluble in saturated NaCl. One animal of the above series (Cow, Dodoma) showed changes similar to those observed by Hartley, but the other three oxen all showed changes similar to those found during immunisation by the simultaneous inoculation of virus and antiserum.

The immunisation of twelve animals has been carried out and the protein changes followed in each beast. In seven animals a steady rise of euglobulin took place until a maximum was reached approximately two weeks after inoculation, followed by a slower decline of this fraction. A reduction of the proportional amounts of pseudoglobulin and albumin took place until the point of maximum euglobulin content, and after this point both these fractions returned to normal. These changes are actually as found by Hartley if protein insoluble in saturated NaCl is considered instead of euglobulin. In three other animals, however, the euglobulin rise took place but there was no reduction of the proportional amount of the pseudoglobulin fraction. Two further animals showed an increase of pseudoglobulin as well as the increase of euglobulin.

These results indicate that although the euglobulin is the globulin fraction most often affected during an attack of rinderpest or during immunisation against rinderpest, nevertheless the pseudoglobulin fraction can also be affected, though to a smaller extent.

Hartley found no increase in the proteins soluble in saturated NaCl (which of necessity are pseudoglobulins) and did not find that this globulin fraction possessed any protective properties. This may be explained by the fact that saturated NaCl precipitates more globulin than the classic euglobulin and contains the least soluble portion of the pseudoglobulin, which is probably also associated with rinderpest antibodies.

The work of Haddow, Sen and Roy[12] indicates that the pseudoglobulin possesses a definite protective action and the above findings support this view.

This idea of antibody occurring in both globulin fractions is more in accord with modern views on the nature of the serum globulins than is the older opinion that the antibody was confined

to one fraction. The work of Chick[16] and of Sorensen[17] indicates that serum globulin consists of an easily dissociable compound of the nature of En Pm and that euglobulin (E) or pseudoglobulin (P) can be separated from the mixture by alteration of the equilibrium point by various salt concentrations. As it is thus doubtful whether euglobulin and pseudoglobulin exist as such in the serum, but are more probably artefacts produced by the precipitation conditions, we should not expect to find a rigid partition of antibody between the globulin fractions. Further, since it is possible to alter the amount of globulin precipitated by increasing gradually the concentration of a salt so that euglobulin with increasing amounts of pseudoglobulin are precipitated, it is easy to see that slight variations of technique or the use of different salts will result in different globulin fractions being estimated.

It has been shown[13, 14] that in various antitoxic serum and also in the antiviral serum for dog distemper[10] the antibody is located on the border-line of the salt precipitation limits for euglobulin and pseudoglobulin. If this is true for anti-rinderpest serum, coupled with the fact that a single precipitation with a salt does not remove euglobulin completely nor entirely free from pseudoglobulin, the occurrence of the antibody in either fraction can be demonstrated by the aid of suitable conditions of separation. The final proof of this distribution of rinderpest antibody has yet to be demonstrated by means of animal protection tests, but the figures obtained by the Muktesar workers and those of this paper suggest that the antibody is associated with the more soluble portion of the euglobulin fraction and with the less soluble portion of the pseudoglobulin fraction. Different methods and conditions of separation will then show slight differences of distribution of the antibody amongst the globulin fractions.

## Conclusions.

(1) Marked changes in the serum proteins of an ox take place during a fatal attack of rinderpest.

(2) These changes are different from those which occur in animals making spontaneous recoveries from the disease, and from those occurring during immunisation of an ox by the simultaneous inoculation of virus and antiserum.

(3) The changes described have been shown to be produced by the virus and not by the experimental conditions, loss of appetite, nor the repeated bleedings.

(4) Antibody development is accompanied by an increase in the globulin content of the serum.

(5) The globulin increase is due most often to a large increase of the euglobulin fraction, though in some animals an increase of the proportions of pseudoglobulin also takes place.

(6) The serum proteins insoluble in saturated NaCl have been shown to be greater in amount than the euglobulin precipitated by 14 per cent. $Na_2SO_4$. These proteins undergo a big increase during immunisation, whilst the globulins soluble in saturated NaCl decrease as the insoluble fraction approaches its maximum value.

(7) A discussion is given of the probable distribution of the antibodies in the serum of immune animals.

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# *CYSTICERCOSIS CELLULOSÆ* IN THE MONKEY.*
## *A CASE REPORT.*

By A. Earl Walker, M.D.

The use in scientific investigation of the primates, particularly the macaque monkey, has increased in recent years. This has enabled us to gain a better knowledge of the diseases with which these animals are afflicted. While those to which the monkey is most susceptible (tuberculosis and dysentery) are well known, the infrequent disorders have received less attention. One of these rarer conditions is the infestation of the animal by the *Cysticercus*. Although in most text-books on parasitology the monkey is mentioned as an intermediate host for the *Tænia solium*, the condition is really quite rare. The medical literature for the past 50 years is silent on cysticercosis in the monkey except for a reference by Vosgien[4] to four cases, one reported in the eighteenth and the others in the nineteenth century. Reports from animal dealers in this country are also to the effect that the condition is rare. The exact incidence will not be known until *post-mortems* are performed routinely on large colonies over a long period of time.

### Case Report.

The subject of the present report was an immature male *Macaca mulatta* bought from an eastern animal dealer. Inquiry of this dealer led to the information that the animals in that lot were from Lucknow, that several " had worms, got very skinny, and eventually died." Apparently the type of worm was not investigated. This animal, except for the fact that he was somewhat emaciated, appeared to be a normal monkey.

A large bone flap was turned down on the left side with the intention of stimulating the cerebral cortex, but upon opening the dura quite unexpected pathology was found. Both in the subdural and subarachnoid spaces were numerous cysts ranging in size from 3 mm. to 15 mm. Those in the subdural space were so loosely attached that when the dura was opened they fell out. Even those in the subarachnoid space enucleated readily when the arachnoid was nicked. On closer inspection, several cysts could be seen within the brain substance, partially covered by the cortex. (Fig. 1.)

After finding this marked pathology in the brain, the animal was killed by an overdose of ether, and a complete *post-mortem* performed. Practically every muscle of the body contained one or

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more of these cysts. The muscles of the back (Fig. 2) had many; those of the extremities (Fig. 3) likewise were studded with cysts. Even the intercostal muscles and the diaphragm had cysts within their parenchyma. Two cysts were found within the heart muscle, one attached to the chorda tendineæ of the left ventricle and the other within the substance of the interventricular septum. None were present within the pericardial sac. The abdominal cavity was likewise free from cysts, but the liver, spleen and both kidneys contained typical cysts. The testes and bladder were normal. The entire gastro-intestinal tract was removed and opened. There was no evidence of any primary worm from which the infection might have arisen, nor was there any other lesion.

FIG. 1.

Photograph of the brain *in situ* to show the numerous cysts both in the subdural and subarachnoid spaces, and in the brain substance. The dura has been reflected upward and folded along the midline.

The cysts when shelled from their bed in muscle, brain or other tissue were all similar. They were slightly translucent. Their walls were composed of a thin transparent membrane, inside which was an aqueous slightly cloudy fluid, which escaped when the membrane was ruptured. Inside the sac of fluid was a small white nodule about 3 mm. in diameter, attached by a broad pedicle to the wall. If the covering of this nodule were incised it was possible to evaginate the scolex, which when examined under a dissecting microscope was seen to have four suckers surrounding the rostellum upon which were mounted a number of hooklets. Each of the cysts presented this same arrangement.

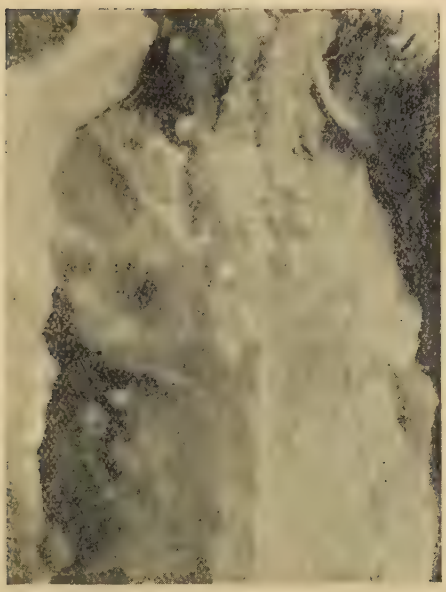

FIG. 2.
Photograph of the posterior shoulder muscles after reflection of the skin to show the cysts.

FIG. 3.
Roentgenograms of the lower extremities to show the massive invasion of the thigh and calf muscles.

Microscopic study of sections taken from the brain, muscle, heart, liver, spleen, kidneys and lungs enables a more complete study of the cyst and its contents, as well as of the reaction of the host. In all the above organs the lack of reaction to the cyst is noteworthy. Except for the displacement and compression of the normal tissues by the cyst, there appears to be little abnormal in these organs. Other than a thin connective tissue capsule about the wall of the cyst there is no inflammatory reaction. There is no evidence of necrosis or softening of adjacent substance such as might be due to toxins produced by the cyst. The vessels adjacent to the cysts show no alterations and there is no perivascular infiltration. Within the brain there is a slight gliosis about the cysts but no other evidence of reaction.

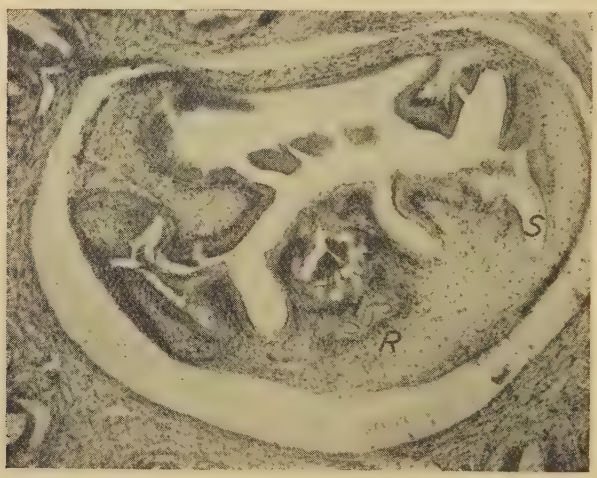


FIG. 4.

Photomicrograph of the scolex showing the sucker pads (S) on either side of the rostellum (R) and the hooklets (H). × 80.

Microscopic studies of sections of the parasite show the structure of the larvæ. The wall is composed of three layers: an outer chitinous epidermis, a middle layer of sparsely spaced cells, and an inner region of loose areolar tissue with many fibres. This latter zone lines the entire cyst cavity, which appears to have been formed by the intracellular fluid splitting the loose areolar tissue.

If serial sections are examined or if a section is cut at a fortuitous plane,[3] the tortuous canal formed by the invagination of the larva can be seen opening into the wall of the cyst. This canal extends to the scolex, which may readily be distinguished by the sucker surrounding the rostellum upon which are a number of hooklets (Fig. 4). The presence of both suckers and hooklets on the scolex serves to define the larva as that of the *Tænia solium*.

## Discussion.

This case, while illustrating the widespread dissemination of the cysticercus, does not present the typical pathological picture seen in man. Although the reaction about the cysts in this case was minimal, severe inflammatory changes are usually seen in cysticercosis cellulosæ in the human. In man a severe meningoencephalitis,[2] leptomeningitis, panarteritis, and cerebral softenings[1] have been described as a result of the invasion of the central nervous system by the parasite.

Since a primary worm could not be found in the alimentary tract of this monkey, it would seem likely that the infection was procured from another animal harbouring the *Tænia solium*. It is, of course, possible that a few small segments and the head of the worm might have been overlooked; however, examination of the fæces revealed no ova, so that an autogenous infection would seem unlikely.

I wish to thank Dr. Jeanette Dean-Throckmorton for assistance in searching the medical literature on this subject.

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## THE ATTEMPTED CURE OF *SCHISTOSOMIASIS* IN STOCK.

By F. Gordon Cawston, m.d. (cantab.).

*Schistosomiasis* in stock would prove to be an insurmountable problem if its control depended on treatment with tartar emetic, for this is effected only by a prolonged series of injections. The success which attended prophylactic measures in dealing with an outbreak at Humansdorp, C.P., in 1928 and 1929, suggested that attempts at curing the sheep might even be discarded. Although I readily obtained the adult parasites from a ram when I visited one of the infected farms in 1929, I am assured that there has been no trouble from the infection since then and that any attempts to obtain further infected material would be disappointing.

This experience indicates that Bilharzia disease in man might as readily be controlled if the necessary precautions were undertaken to prevent infection; even as the disease was suitably controlled in Japanese oxen by substituting horses, which are relatively immune, for ploughing in a badly infected district.

The disease is likely to assume added importance in Mediterranean lands as a result of the movement of troops and the use of animal transport, and this may result in the discovery of new schistosomes to add to those reported since Montgomery[3] published his standard work on the subject.

Under the Streatfield Research Scholarship, 1919 to 1922, I showed that antimonium potassium tartrate was effective in destroying the Chinese schistosome as well as the three found in man in South Africa. P. L. Le Roux[2] reported some interesting facts about South African sheep receiving repeated injections of tartar emetic. Veterinarians rightly prefer this drug to the sodium equivalent, which is less stable under tropical conditions.

It is suggested that the cure of schistosomiasis will ultimately be achieved by one very potent injection or by the discovery of a drug which may be administered orally. Dr. H. H. Curson's experience with tartar emetic in the treatment of Zululand cattle suffering from nagana rather suggests that the treatment will be effected by a more limited number of skilfully administered injections.

On a recent visit to the Zululand Game Reserve I found that farmers preferred to administer their own injections, with the result that the solution was made up in distilled water, not necessarily sterile, and that a local abscess commonly occurred. It is suggested that local sepsis might be controlled by the use of dilute phenol instead of distilled water, as employed with tuberculin injections.

Both phenol and glycerine have been used to lessen local reaction from intramuscular injections of tartar emetic in man. The practice might be applied for injections where there is much

danger of a few drops of an irritating solution escaping out of the vein or into the vein wall.

Considerable advance has been made in the cure of schistosomiasis in man by the introduction of the oxyquinoline derivatives of antimony. These enable a cure to be effected in considerably less time than is required with tartar emetic and other popular antimony compounds.

It is suggested[1] that these newer derivatives, if proved to be applicable to animal diseases, should render the cure of schistosomiasis of stock a more practical one. Stibilase has a far higher antimony content than Antimosan which is employed in the treatment of cattle in Zululand, and the solution is non-irritating, should a few drops escape during an intravenous injection. The addition of glycerine and carbolic acid would also enable larger doses of its allied preparation Trystibine to be administered subcutaneously, if under any circumstances an intravenous injection is contra-indicated.

Investigations would seem to show that both Stibilase and Trystibine, which have been used by the Belgians for the treatment of trypanosomiasis and schistosomiasis, are more potent in the latter disease.

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# NOTES ON A MINOR OUTBREAK OF CELL INCLUSION DISEASE.

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The minor outbreak of cell inclusion disease now described occurred in the vicinity of Jaffa during a period of a few weeks in June, 1935. About this time a number of dead fowls forwarded from different poultry farms showed inclusion bodies in the blood, but from none of these cases was it possible to transmit a fatal form of the disease. The mortality observed in the outbreaks was variable, as is shown by the following table :—

| *Farm.* | *Number of Flock.* | *Died.* | *Mortality.* | *Duration of Outbreak.* |
|---|---|---|---|---|
| 1 | 180 | 35 | 19·5 per cent. | 20 days |
| 2 | 130 | 115 | 88·5 „ | 10 „ |
| 3 | 200 | 10 | 5 „ | 32 „ |
| 4 | 200 | 6 | 3 „ | 10 „ |

Subsequent to this period no further outbreaks were reported.

Owing to the difficulty in transmitting the disease in a fatal form it was decided to concentrate on Farm No. 3, an agricultural school adjacent to Jaffa. The disease had been observed at this school in four adjoining concrete yards each containing about 50 fowls. The method adopted was to raise the virulence by rapid passage, which had been found successful on previous occasions.

Inoculated fowls were killed 24 hours later when showing high temperatures and inclusions in the leucocytes on microscopical examination of the blood. Four such passages were found to produce a virus of fatal virulence, the period of incubation varying from one to five days. Subsequent passages to maintain the virus showed a constant feature of inclusions in the leucocytes and a temperature of 110° F. or higher occurring within 24 hours.

*Tenacity of the Virus.*

It has been consistently observed that cell inclusion virus does not cause the disease if preserved in 50 per cent. glycerine-saline for more than ten days, or at least only very exceptionally.

In order carefully to re-check this finding the following experiments were carried out :—

Spleen and liver material was collected from a fourth passage fowl killed 24 hours after inoculation and preserved as follows :—

(*a*) Whole liver and spleen in 50 per cent. glycerine and saline.

(*b*) Liver and spleen minced with a knife into as small pieces as possible and preserved in 50 per cent. glycerine and saline.

(*c*) Liver and spleen pulped in a mortar (as prepared in usual inoculations) but added to 4 parts of 50 per cent. glycerine and saline instead of saline alone.

These materials were placed in the refrigerator at about 40° F. for 14 days and then 1 c.c. of each was inoculated intramuscularly into one fowl in each case. A fourth fowl received a similar amount of fully virulent fresh virus as a control.

None of the first three fowls reacted except for a slight rise of temperature next morning. The fowl inoculated with whole material (*a*) also showed a few inclusions in the blood. The control fowl showed the usual symptoms of the virulent disease and died five days after inoculation. These results correspond with our previous findings as regards the tenacity of cell inclusion disease virus when preserved in glycerine saline, and the method of preserving the material does not appear to have affected the issue.

## Discussion.

The variability of mortality and the low tenacity of the virus of cell inclusion disease has again shown itself in the minor outbreak now described.

Komarov (1934) has described his work on a virus taken from a locality in which cell inclusion disease was found by us to be prevalent at the time. With a passaged virus of considerable virulence he proceeded to show that it was identical with fowl plague by means of cross-immunity tests. Whatever might prove to be the technical relationship between this disease or other possibly aberrant types, the practical outlook cannot be ignored. Cell inclusion disease has been a matter of regular recurrence since it was first described as occurring in Palestine by Adler (1925). The total mortality from this source, however, has been small. In spite of this fact it has never, with one possible exception, developed into an outbreak of typical plague. This exception consisted of the fowl plague outbreak of 1928 which could not have been mistaken for anything else. On this occasion plague spread rapidly from the Egyptian border throughout Palestine, with a total estimated mortality of 45,226 head, or 73·2 per cent. of the birds which were reported to have become infected. As fowl plague is indigenous in Egypt, the possibility of the outbreak having extended from that country appears more than probable. During this outbreak blood slides were examined on many occasions but leucocyte inclusions were never observed. It was only eradicated by severe administrative measures such as complete stoppage of movement of poultry, etc. To apply such measures to outbreaks like those herein described would be as unpractical as it would be unnecessary. This is all the more so when

it is considered that cell inclusion disease presents such distinctive features of its own as have been shown by us previously (1934) and that these features have been constant for more than ten years. The association of the inclusions has been most definite in our experience and they have certainly not been found to be a normal association of spirochætosis as stated by Komarov (1934). The authors are of the opinion that until better grounds can be shown for not separating this disease from plague, there appears to be every reason why it should continue to be identified under its present name.

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# THE TREATMENT OF EQUINE *TRYPANOSOMA CONGOLENSE* INFECTIONS WITH SURFEN C (BAYER).

By S. C. J. Bennett, D.SC., M.R.C.V.S.
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Surfen C (Bayer) is a preparation that has very recently been introduced for the treatment of infections with the *T. congolense* type of trypanosome. It is, in fact, claimed by the makers that this drug is especially active against the small monomorphic trypanosomes and relatively useless for the treatment of other types.

Although the preparation is said still to be in an experimental stage, and it is not yet commercially available, a number of references have already been made to it in recent tropical African veterinary literature. These references have been to its value particularly in the treatment of bovine *T. congolense* infections, and, although no very extensive work has yet been recorded, the general opinion is that this drug is capable of effecting a very high proportion of cures following the administration of a single dose at the rate of 1 gm./100 kg. body weight. A small series of trials carried out in the Sudan has led to a similar conclusion. Thus, even at the present early stage, and with no other dosage investigated beyond that suggested by the manufacturers for preliminary study, it is evident that Surfen C has shown itself to be the most promising preparation yet introduced for treating bovine infection with *T. congolense*. For general field use its most attractive feature is the very high probability of establishing a single dose technique of treatment. The subject of this short paper, however, is equine and not bovine trypanosomiasis, and the latter need not be further discussed, but the foregoing preliminary remarks were necessary for subsequent appreciation of the different behaviour of horses and cattle when treated with Surfen C.

The subject matter of this paper is provided by 14 horses, all naturally infected with *T. congolense* in tsetse areas. In some of these the original diagnosis was effected by seeing trypanosomes in the blood, while in others resort was made to small-animal inoculation. It is necessary to add that even in the cases diagnosed by direct blood examination the transmissibility of the trypanosome to small animals was confirmed, since sub-inoculation was projected as a means of checking apparent cures. For one's own part this precaution seemed superfluous, since no strain of *T. congolense* that the writer has tested in the Sudan has ever failed to infect small animals, provided the inoculum was known to contain living trypanosomes. Nevertheless, with the recollection that so-called *T. nanum*, reputedly not transmissible to small

animals, had in the past been said to occur in the Sudan, lack of proof of transmissibility might arise as a criticism if it were not confirmed.

The object of these studies was to establish, if possible, a single dose treatment. The manufacturers suggested that 1 gm./100 kg. should be a suitable dose, and it was decided to start by trying this quantity on a number of horses. It was proposed that, if failures were encountered among the earlier treated cases, higher single doses should then be tried, and further, so far as circumstances permitted, in any case in which the first dose had failed to effect a cure, a sufficiently long period should be allowed to elapse before giving a further injection for all residual effect of the first dose to have disappeared. Actually whenever possible two months was the period allowed, since this was comfortably longer than that claimed by the manufacturers as the longest over which prophylactic action should be appreciable. In view of this procedure each re-treatment may be provisionally regarded as a fresh experiment with a single dose, and not part of a multiple dose course.

Regarding the technique of injection, the manufacturers state that the drug is in general unsuitable for intravenous or subcutaneous administration, and recommend the intramuscular route, using solutions not stronger than 2·5 per cent. In the absence of records of equine treatment it was thought advisable to check this recommendation. As will be seen from the protocols which follow, the advice is applicable to horses.

All the horses treated in this series were native Sudani (beladi) ponies of fairly standard size, none varying much from a weight of 250 kg. For convenience, therefore, they were all regarded as being of this weight and 100 c.c. of a 2·5 per cent. solution of drug reckoned as a dose of 1 gm./100 kg.

For reasons which will presently become apparent, the best way of recording one's experiments will be to describe each case separately and then to discuss the results. In order that each case may be rapidly assessed, a note of quantities of Surfen C injected will precede each report. The fact that more than one dose is recorded indicates that failures occurred with the earlier ones (with the exception of Case No. 1).

*Case No.* 1 (*Horse A.S.*) ? 2+2·5 *gm.*

Marked clinical symptoms. Diagnosis confirmed by direct blood examination, although *T. congolense* was only seen twice in 13 days.

26.6.34. Attempted to give 100 c.c. intravenously. After 50 c.c. had run in the horse became uneasy and at 70-75 c.c. collapsed, ceased breathing and pulse became imperceptible. After about ten minutes, however, he was on his feet again, though very depressed.

12.7.34. Daily blood examination to date negative. Two gerbils (jumping desert mice, highly susceptible to trypanosomes) each given 0·5 c.c.

blood intraperitoneally (these remained healthy). Horse given 100 c.c. Surfen C solution (2·5 gm.) subcutaneously in two 50 c.c. doses on either side of the neck. Raised swellings about 20 cm. diameter developed, and as these did not resolve within a month they were surgically treated. The contents of the swellings appeared to be pure serum.

15.8.34. Daily blood examination negative to date. Two gerbils each given 0·5 c.c. blood. These remained healthy.

15.9.34. Gerbil inoculation repeated, again with negative results. Horse much improved in condition and discharged as cured. Report one year later that horse was in good condition and playing polo.

*Case No.* 2 (*Horse I.*109) 2·5 + 3·75 *gm.*

Marked clinical symptoms, *T. congolense* seen in blood almost daily.

12.5.35. Given 2.5 gm. (100 c.c.) Surfen C intramuscularly, half into each thigh. Both thighs swelled appreciably but were not very painful. Swelling reduced by light walking exercise but reappeared later until the lapse of twelve days.

12.6.35. Horse improved. Daily blood examination negative to date. Two gerbils injected with 0·5 c.c. These developed trypanosomiasis.

14.7.35 (two months after first treatment). Given 3·75 gm. (150 c.c.) Surfen C, half in each thigh. Resultant swelling no more troublesome than before.

14.8.35. Blood examination negative to date. Two gerbils inoculated. These remained healthy. Horse discharged. Report five months later that horse had worked continuously and was in good condition.

*Case No.* 3 (*Horse* 141.*H*) 3·75 *gm.*

Marked clinical case with trypanosomes in blood.

26.5.35. Given 3·75 gm. (150 c.c.) Surfen C as four intramuscular injections, into both thighs and buttocks. Considerable swelling of thighs but less of buttocks followed, and all ultimately cleared up with light exercise.

5.6.35. Blood negative to date. Horse developed strangles which developed into fatal streptococcic septicaemia.

*Case No.* 4 (*Horse F.*145) 3·75 *gm.*

Clinical symptoms, trypanosomes in blood one day out of eight examined.

23.5.35. Given 3.75 gm. (150 c.c.) Surfen C as in preceding case. Some swelling followed, the greater of which was in the gluteal site (reverse of Case No. 3). Swellings resolved with a few days' exercise.

23.6.35. Horse improved. Blood examination negative to date. Two gerbils injected, which remained healthy. Horse discharged. Report seven months later that horse was in good condition and at work.

*Case No.* 5 (*Horse I.*11) 2·5 *gm.*

Clinical symptoms, with trypanosomes in blood.

2.6.35. Given 2·5 gm. (100 c.c.) Surfen C in four doses intramuscularly as above. Very little swelling at any site of injection.

8.7.35. Horse improved. Blood examination negative to date. Two gerbils injected, which remained healthy. Report six months later that horse was at work and in good condition.

*Case No.* 6 (*Horse I.*50) 2·5 + 3·75 + 5·0 *gm.*

Clinical symptoms, *T. congolense* in blood.

2.6.35. Given 2·5 gm. (100 c.c.) Surfen C in four sites as above ; resultant swellings negligible.

8.7.35. Horse improved in condition. Blood examination negative to date. Two gerbils injected, both of which developed trypanosomiasis.

15.7.35. Given 3·75 gm. (150 c.c.) Surfen C in four sites as above. Swellings developed in the thighs only, but soon resolved.

14.8.35. Horse continuing to improve in condition. Blood examination negative to date. Two gerbils injected. Both these developed trypanosomiasis.

28.8.35. Given 5·0 gm. (200 c.c.) Surfen C, which was followed by swellings very slightly more troublesome than after 150 c.c.

28.9.35. Improvement in condition maintained. Blood examination negative to date. Two gerbils injected, both of which remained free. Horse was kept under observation for a further six weeks, during which it rapidly improved in condition. Discharged without further gerbil inoculation and reported five months after last treatment as working and in good condition.

*Case No.* 7 (*Horse I.*97) 2.5 *gm.*

Clinical case with trypanosomes in blood.

2.6.35. Given 2·5 gm. (100 c.c.) Surfen C in four injections as above. Slight swellings resulted in thighs but not in buttocks.

8.7.35. Blood examination negative to date. Two gerbils injected, which remained healthy. Horse discharged, and reported six months later as working and in good condition.

*Case No.* 8 (*Horse I.*214) 2·5 *gm.*

Same treatment and same observations on same dates as preceding case. Results also in every way similar.

*Case No.* 9 (*Horse I.*55) 3·75 + 5·0 + 7·5 + 12·5 *gm.*

Clinical case with trypanosomes in blood.

13.7.35. Given 3·75 gm. (150 c.c.) Surfen C in usual four sites. Very little swelling followed.

14.8.35. Horse still very poor although blood examination constantly negative. Two gerbils injected, both of which developed trypanosomiasis.

9.9.35. Given 5 gm. (200 c.c.) Surfen C which was followed by very little swelling.

9.10.35. Horse improved somewhat in condition, but still poor. Blood examination constantly negative. Two gerbils injected, both of which developed trypanosomiasis.

9.11.35. Given 7·5 gm. (200 c.c.) Surfen C. It will be seen that in order to avoid injecting a greater bulk of solution the drug was given in slightly more concentrated form than before. Large swellings developed, and although these never gave any fear of suppurating they were at any rate troublesome.

12.12.35. Earlier improvement in condition not maintained, but blood examination always negative. Two gerbils injected, both of which became infected.

Having failed to cure this horse with doses of up to 7·5 gm., and having further found that to give a more concentrated solution than 2·5 per cent.

caused troublesome swelling, it was decided to give 12·5 gm. in five weekly doses of 2·5 gm. each, the object being to allow the slight swelling that would develop from one dose to subside before the next was given. Thus :—

7.1.36-8.2.36. Given 12·5 gm. Surfen C in five weekly injections of 2·5 gm. (100 c.c.). The horse appeared at first to withstand these injections as well as it had withstood the earlier ones of this size, but a few weeks later some diffuse swelling occurred in one thigh which took about six weeks to disappear.

8.3.36. Condition improved, and blood negative to date. Two gerbils injected, both of which survived.

14.3.36. Injected 50 c.c. blood into a yearling bull, which remained healthy and free from trypanosomes.

*Case No.* 10 (*Horse* 11) 3·75 *gm.*

Clinical case with trypanosomes in blood.

13.7.35. Given 3·75 gm. (150 c.c.) Surfen C in usual four sites. No swelling whatever developed.

14.8.35. Horse improved. Blood examination constantly negative. Two gerbils injected, which remained healthy. Horse discharged and reported fit and working well six months after treatment.

*Case No.* 11 (*Horse I.*34) 3·75 *gm.*

Doubtful clinical symptoms; had been exposed to infection but blood examination constantly negative. Two gerbils were injected, both of which showed *T. congolense.*

13.7.35. Given 3·75 gm. (150 c.c.) Surfen C in usual four sites. Negligible swelling resulted.

14.8.35. No notes on improvement in horse, as it was never in less than fair condition. Blood examination constantly negative. Two gerbils injected with negative results. Report six months after treatment that horse was in work and in good condition.

*Case No.* 12 (*Horse I.*40) 3.75 *gm.*

Clinical symptoms not marked, no trypanosomes in blood. Infection diagnosed by positive gerbil inoculation.

13.7.35. Given 3·75 gm. Surfen C (150 c.c.) in usual four sites. Only one thigh developed slight swelling which soon resolved.

27.7.35. Horse developed strangles.

14.8.35. Condition lost on account of strangles infection. Blood examination constantly negative. Two gerbils injected which remained healthy. Six months after treatment horse reported as working and in good condition.

*Case No.* 13 (*Horse I.*152) 5.0 *gm.*

No trypanosomes in blood although persistently examined for over a month. Diagnosis effected by inoculation of gerbils.

13.7.35. Given 5 gm. (200 c.c.) Surfen C as above. Slight swelling of thighs resulted, but no swelling of buttocks.

14.8.35. Slight improvement in condition, and blood examination negative to date. Two gerbils injected with negative results. Six months after treatment horse was in full work and in good condition.

*Case No.* 14 (*Horse I.*9) 5·0 + 7·5 *gm.*

Clinical symptoms and trypanosomes in blood.

13.7.35. Given 5 gm. (200 c.c.) Surfen C as above. No swellings resulted.

14.8.35. Condition of horse not improved, but it had meanwhile developed " bastard " strangles which reduces the significance of this observation. Blood examination constantly negative to date. Two gerbils injected with negative results.

11.11.35. Horse still not improved in condition, but thought to be due to the severe strangles infection. Two more gerbils injected, again with negative results.

20.1.36. Trypanosomes in horses' blood.

20.1.36-8.2.36. Given 7·5 gm. Surfen C as a series of three injections of 2·5 gm. (100 c.c.) at intervals of ten days. The horse appeared to withstand these injections fairly well, but about a week after the last one considerable swelling developed in one buttock, extending in front of the angle of the ilium. This swelling later condensed into two relatively painless fluctuating circumscribed swellings one anterior to and the other posterior to the angle of the ilium. These showed no signs of disappearing at the end of a month and had to be opened ; the contents resembled pure serum.

8.3.36. Horse in moderate condition. Two gerbils injected, both of which developed *T. congolense*. It was judged from the indifferent manner in which the patient had withstood a well distributed dose of 7·5 gm. of the drug that further observation of the case would provide no fresh information. A large dose would probably not have been fatal, and might even have effected a cure, but the general issue would not have been affected. Destruction was therefore ordered.

## Discussion.

Before discussing efficacy it is as well to dispose of the question of safety. Surfen C is certainly not safe to give to horses intravenously. The only horse so treated was almost certainly cured by the intravenous injection of 2 grammes, as seen by the survival of gerbils injected with its blood three weeks later, but the symptoms produced by the treatment were altogether too alarming for intravenous administration to be further studied. It is equally certain that the subcutaneous route is unsuitable, even though not so dangerous. Large persistent swellings calling for surgical treatment developed in the only case studied, although no undesirable systemic symptoms developed.

The intramuscular route is undoubtedly the one to choose, as shown by the remaining 13 cases. In the horses which only had to be given 2·5 gm. or 3·75 gm. local swelling certainly developed, but this never gave rise to any anxiety or trouble, being soon resolved with a little light exercise. With larger doses, however, the sequelæ were at least troublesome if not actually objectionable. For example, in Case 6, which received only 5 gm. in 2·5 per cent. solution, the swellings were larger and more persistent than those following 3·75 gm. two months earlier in the same patient. Again, Case 9, which withstood 3·75 gm. and 5 gm. quite well,

developed rather troublesome swellings after 7·5 gm. in slightly more concentrated solution. Even in Cases 9 and 14, in which series of relatively small spaced-out doses were given, the injections led to very troublesome swellings; in Case 14 calling for surgical treatment. It is particularly to be noted, on the other hand, that in no case did undesirable systemic (toxic) sequelæ develop.

The evidence on the count of safety may thus be roughly summarised by saying that intramuscular injection is " safe " in the generally accepted chemotherapeutic sense of the word, but is nevertheless apt, especially with more than minimal doses, to be troublesome; the trouble, moreover, cannot be avoided either by dividing the dose into small portions and injecting at multiple sites or by spacing out the doses and allowing a short interval of time between injections into any site.

Curative efficacy of Surfen C is a quality that in the case of equine *T. congolense* infection has to be discussed in even more relative terms than safety. The feature with the widest practical implications is the extremely variable response of individual horses, this being quite different from what occurs in cattle. In the latter, judging from such observations as have been recorded, a single dose of 1 gm./100 kg. cures the great majority of patients. Larger doses have not yet been studied, but it will be interesting to see whether a single dose of convenient size will not cure practically all of them, as happens for example with Naganol in *T. evansi*, and perhaps also *T. brucei* and *T. vivax*, infections in camels. In any case, cattle infected with *T. congolense* appear to react much more uniformly than horses to Surfen C.

As already explained, it is permissible to examine the foregoing protocols in the light of the assumption that repeat injections are fresh treatments. The manufacturers report that in a dog a dose of 25 gm./kg. (two and a half times the recommended therapeutic dose) " still evidenced considerable prophylactic action against *T. congolense* infection after three weeks." One gathers from the wording that this " considerable prophylactic action " fell short of complete protection. In sheep and goats 20 mg./kg. is said to protect for from four to six weeks. One is therefore fairly safe at this early stage of study in assuming that in the horse, in which the reaction to *T. congolense* resembles that in the dog rather than that in the sheep, no appreciable protection would remain after an interval of two months following doses of 2-3 gm./100 kg. One of the four horses in the foregoing series which received more than a single dose of the drug (Case No. 6) was re-treated at intervals of only six weeks but, as the second dose was no more successful than the first, the argument would apply even more forcibly in this case.

In the twelve horses followed up—neglecting Cases 1 and 3—19 single dose treatments can be regarded as under examination.

The results are most conveniently studied in tabular form as follows :—

| *Dose, gm./100 Kg.* | 2·5 | 3·75 | 5·0 | 7·5 | 12·5 |
|---|---|---|---|---|---|
| Cures ... ... ... ... | 3 | 5 | 2 | 0 | 1 |
| Failures ... ... ... ... | 2 | 2 | 2 | 2 | — |

A brief glance at this small table shows that the wide variation in response on the part of individual horses entirely precludes any hope of instituting some form of single dose mass treatment, or for that matter, any other form of "routine" treatment. Nevertheless, as the drug was "safe" in the sense that it produced no undesirable toxic symptoms, although its administration was followed by other troublesome developments, so it was "efficacious" in the sense that it apparently cured twelve out of the 13 horses treated with it (neglecting Case 3, which died of intercurrent disease).

A further most important consideration from the practical standpoint is the methods that must be adopted to confirm apparent cures. Examination of the patient's blood is for practical purpose useless, since one may examine it daily for months and find no trypanosomes, although the patient may still be infected. Improvement in general condition as an indication of cure may also be immediately dismissed from account. Any form of treatment—and there are many besides the injection of Surfen C—which will temporarily check infection may allow a patient to recover general bodily condition, and maintain it until the next relapse supervenes. Conversely, some individuals, although genuinely cured, may take several months to recover their normal strength. Small-animal sub-inoculation greatly increases one's chances of detecting latent infection, but this test, which is merely the examination of a larger volume of circulating blood than can conveniently be examined with a microscope, may occasionally fail. If this test were only to be used for confirming the results of experimental treatment, and if following its use a more or less fixed routine treatment could be evolved, rendering sub-inoculation as a check to cures unnecessary in general practice, then occasional failure to detect uncured patients would no longer matter. The foregoing series of cases, however, demonstrates that no such routine treatment is likely to be developed in horses—unless one is prepared to treat every case with perhaps five or six times as much Surfen C as will cure a good many of one's patients, with troublesome local swellings as an added disadvantage.

## Conclusions.

The conclusions that may be drawn from the foregoing evidence and discussion are as follows :—

(1) It is almost certainly unsafe to administer Surfen C to horses intravenously.

(2) While "safe" in the usual chemotherapeutic sense to inject it subcutaneously, large swellings developing at the site of injection render this route objectionable.

(3) Intramuscular injection is less objectionable than subcutaneous, although even by this route, in spite of giving multiple small doses, or even of giving a series of doses at intervals of a week or ten days, troublesome local swellings may develop.

(4) In no case in the recorded series of treatment did any toxic symptoms develop following intramuscular injection, indicating that horses can, apart from possible surgical complications, probably tolerate appreciably larger doses than were used in this series of observations.

(5) Surfen C is certainly capable of curing nearly all, if not all, cases of *T. congolense* in horses, and in this respect it is the best agent hitherto studied. Its only drawback—and it is a serious one when the necessity for routine mass treatment has to be contemplated—seems to be referable not so much to the drug as to the patient, namely, the extreme variability of individual response. Some horses are cured by a single dose of 1 gm./100 kg. while others require much more. It is therefore unlikely that any orthodox course of treatment will be developed; each patient will call for close individual attention, even to the degree of confirming cure by small-animal sub-inoculation.

Acknowledgment.

I gratefully acknowledge the help of my Laboratory Assistant, Mr. P. A. C. Kenny, who, owing to my absence from Khartoum, was in sole charge of these observations from late July to late October 1935.

# TRYPANOSOMA CONGOLENSE INFECTION IN CATTLE: TREATMENT BY ANTIMOSAN AND SURFEN "C."

By J. T. R. Evans, B.Sc., M.R.C.V.S.,
*Assistant Veterinary Research Officer, Sudan Government.*

Trypanosomiasis due to *T. congolense*, although a widespread disease amongst cattle in Africa, is confined to comparatively small, well-defined areas in the Sudan, and is, generally speaking, one of the country's minor problems. Its significance, however, has increased during recent years owing to its appearance amongst bulls used for the production of cattle plague antiserum at Malakal. For various reasons the majority of the animals used for this work are obtained from that part of the Sudan which borders on Abyssinia in the region of parallel 9° N. No entomological survey of this land has been carried out, but tsetse areas have been encountered, and it is known that as the land rises into Abyssinia *Glossina morsitans* become increasingly common. When infected animals come to the laboratory their vitality becomes lowered owing to the effects of hyperimmunisation and bleeding, and, as would be expected, trypanosomiasis soon manifests itself in those cases in which the infection lay dormant.

The following small experiments on the treatment of this disease were carried out in conjunction with the production of cattle plague antiserum. The annual demands for serum have been heavy, and, in order to satisfy these as much as possible, all apparently recovered animals were turned into serum producers as soon as the improvement in their general condition warranted the change.

## I.—Antimosan.

In 1934 a very rough experiment was carried out, using Antimosan (Bayer) as the therapeutic agent. The main objective was not so much to determine whether Antimosan would effect a complete cure as to ascertain whether treatment with this drug would be an economic procedure for treating infected serum producers.

Twenty-seven bulls, diagnosed as being infected with *T. congolense* by microscopic examination, were given intravenous injections of Antimosan. Twenty of them received a course of five injections at weekly intervals, whilst the remaining seven were given three injections at similar intervals. The dose in each case was the minimum recommended (40 c.c.), and the weights of the animals lay between 700 lb. and 800 lb.

It was not possible to check apparent cures by small animal inoculations, and the effect of the treatment was judged, apart from microscopic examination of the blood, by any changes which followed in the general condition of the bulls, and their ability to withstand routine hyperimmunisations and bleedings.

The results were as definite as could be expected from such a roughly-designed experiment. All the bulls which received five injections improved in condition, and half of them maintained that improvement during succeeding hyperimmunisations and bleedings. The other half gradually became weaker as a result of these operations and were bled to death whilst their serum was still of value. None of the cattle that received only three injections showed any improvement. It seems, therefore, that whether or not a " full course " of five injections would effect a cure, less than five injections could certainly not be relied upon even to retard the progress of the infection.

Antimosan was given a more intensive trial in 1935, and sub-inoculation tests on small animals were carried out with the bloods of the apparently cured cases.

Twelve bulls, previously diagnosed by microscopic examination as being infected with *T. congolense*, were each given a course of five intravenous injections of Antimosan at intervals of a week. The dose in each case was 10 c.c. per estimated 100 lb. body weight, and the average for the group was 60 c.c. The first injections were given on February 12th, 1935, and the last on March 12th, 1935. Microscopic examination of blood taken from each bull at approximately weekly intervals from March 26th, 1935, onwards failed to show the presence of any trypanosomes. On June 11th, 1935, 0·5 c.c. of blood from each bull was injected intraperitoneally into each of two gerbils (desert mice susceptible to *T. congolense*).

The results were fairly definite. Two gerbils, from separate pairs, died on the third and fourth morning respectively after injection, and need not be taken into account. The remainder survived, and no trypanosomes could be found in blood smears taken from them from the seventh to the fourteenth day after injection. The group of treated cattle was under observation for four months, and at the end of this period they were used for routine serum production and shortly afterwards bled to death when the laboratory closed down for the season. The majority of the bulls improved in condition after the treatment. Those that did not were all old bulls, and in these no further emaciation was noticeable.

This second small experiment showed that Antimosan, provided a full course of doses of the size recommended by the manufacturers be given, is of the highest value in treating *T. congolense* infection in cattle.

## II.—Surfen " C."

This small experiment was designed to observe the therapeutic action of Surfen " C " (Bayer) in bovines infected with *T. congolense*. The drug was supplied in the form of a 2·5 per cent. solution, the dose recommended for a bull being 100 c.c.

Seven bulls, in which *T. congolense* had previously been demonstrated in the blood, were treated on March 8th, 1935. The dose (100 c.c.) was divided into two parts and injected slowly into the thigh muscles of each leg. No local or systemic reactions followed the injections.

One bull, which was in very poor condition when treated, died 13 days later. Examination of blood smears taken shortly before death failed to show the presence of any trypanosomes. The remaining bulls were kept under observation for three-and-a-half months after treatment, by the end of which period they had all improved considerably in condition, were turned into serum producers, and ultimately bled to death. Two gerbils each were given 0·5 c.c. of blood intraperitoneally from each of the six surviving bulls on May 6th, 1935 (approximately two months after treatment). All the gerbils survived and no trypanosomes could be found in blood smears taken from them from the seventh to fourteenth day after injection.

## Conclusions.

In spite of the small number of animals treated with either of the drugs under consideration, it is permissible to draw the following conclusions:—

(1) In the first experiment the failure to check apparent cures by sub-inoculation was of no account, since on clinical grounds alone it was evident that any course of Antimosan less intensive than that recommended by the manufacturers would offer no hope of success.

(2) In the second experiment, fairly lengthy observation of the treated cattle and the sub-inoculation of small animals were a far more effective check, following which one may conclude that a full course of Antimosan treatment as recommended by the manufacturers offers great hope of a complete cure being effected.

(3) The third experiment, with similar precautions to check apparent recoveries, indicates that a single dose of Surfen " C " offers as high a hope of effecting a complete cure as does a full course of Antimosan treatment.

It is realised that, had circumstances permitted, the period over which the patients were observed could profitably have been prolonged; sub-inoculation tests could also have been repeated. Nevertheless, the promising results obtained with a single dose of Surfen " C " mark this drug as the one which should receive further study, since treatment of bovine *T. congolense* infection in the field will, under Sudan conditions, not be practicable unless some single dose system of treatment is available.

These experiments were carried out at the suggestion of Dr. S. C. J. Bennett, Veterinary Research Officer, Sudan Government, to whom I am indebted for much helpful advice.

# THE LIFE-HISTORY OF *PSOROPTES COMMUNIS* VAR. *OVIS* WITH PARTICULAR REFERENCE TO LATENT OR SUPPRESSED SCAB.

By W. Downing, f.r.c.v.s.

## I.—Introduction.

Sheep scab is a contagious skin disease of sheep, caused by the activities of the acarine parasite, *Psoroptes communis* var. *ovis*. The disease is characterised by an acute irritation, which causes the sheep to bite itself, rub against posts, scratch with the hind legs during the active phase of the disease, and to show its gratification, when the affected part is handled, by turning its head round and smacking its lips in a characteristic manner. As the disease progresses the sheep gradually loses condition, due to lack of rest and secondary septic infections, and death may result from debility and exhaustion. Sheep heavily infested with scab are sometimes subject to fits.

The lesions of the skin are produced by the feeding of the acari. At the point where the acarus has fed, a small vesicle is formed, filled with a clear lymph and surrounded by a zone of inflamed skin, which presents a moist greasy appearance due to serous discharge which causes a matting of the surrounding wool. As a result of infection by pyogenic bacteria, the vesicles often become pustules of a greenish-yellow colour. Later, crusts are formed by disintegration of the cuticular layers of the skin and the accumulation of serum and purulent matter derived from the ruptured vesicles and pustules. The wool over the affected parts is often ultimately shed and sores are produced by the rubbing and scratching.

It is on the moist inflamed advancing margin of the lesion that the acari are usually found. They may also be found, to a lesser extent, in amongst the moist crusts, but rarely in the dry crusts. As the disease develops, the acari tend to migrate from the initial lesion and to start new centres of infection. In the first instance the eruption is confined to the dorsal and lumbar areas of the skin, but if treatment is deferred it may extend over the whole body surface down the flanks and limbs and also on to the head, face and tail.

Sheep scab is one of the oldest known diseases of sheep, but it is only during the last hundred years that the causative parasite has been identified and its life-history studied. The first observations of the *Psoroptes communis* var. *ovis*, as the causative parasite of sheep scab, was made by Walz, G. H. (1809),[1] who described and illustrated the mite. Other authors were Hering (1835),[2] Hertwig (1835)[3] Delafond and Bourguignon (1854),[4] Gerlach (1857),[5] Stockman (1909),[6] (1910),[7] (1911)[8] and Stockman

FIG. 1.
Scab-infested sheep.

FIG. 2
Advanced cases of sheep-scab, showing extensive depilation.

and Berry (1913),[9] but the most comprehensive work was that of Shilston in South Africa in 1915.[10]

There is, however, an aspect of the disease which up to the present has failed to attract the attention which it deserves, in view of the importance which it possesses in relation to control and eradication of the disease. For a number of years past, the writer and his colleagues have observed that sheep which have been heavily infected with psoroptic scabies in the winter and which have been left untreated, undergo, during the summer months, an almost complete recovery, only to relapse in the autumn. This

Fig. 3
Another advanced case.

apparent recovery has also been recorded by Stockman (1909)[6] and Shilston (1915).[10] They ascribe its occurrence as being due to shearing, improved pasture and condition of the sheep, and the presence of an increased proportion of grease in the fleece. It was with the idea of attempting to elucidate this problem of latency or suppressed scab that the experiments recorded below were commenced by the writer in December 1932.

## II.—Life-History.

### (1) *Methods.*

To facilitate observation of the successive phases of the life-history, it is necessary to confine the acari to a restricted area. With this object in view the following technique was evolved.

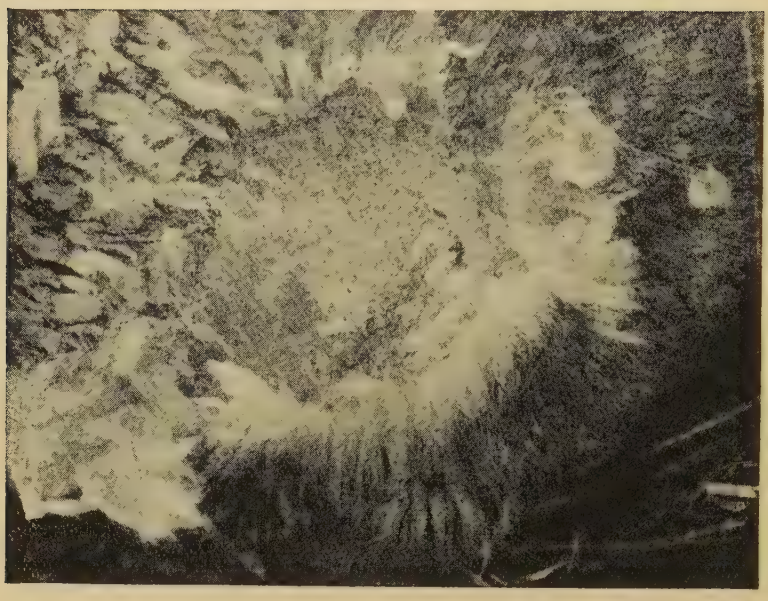

FIG. 4.

Scab lesion, showing inflamed marginal zone, with acari.

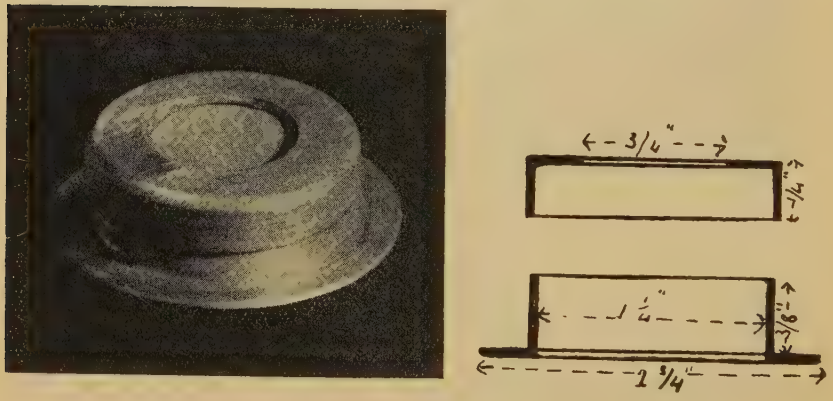


FIG. 5.

(*a*) Aluminium culture cell for raising acari on sheep under observation. (*b*) Diagram of cell with dimensions.

Aluminium cells of the design illustrated in Fig. 5 were made to my specification by Messrs. Z. Dessar & Sons, Ltd., 5, 6 and 7, Museum Street, W.C.1, for this purpose. The large opening in the lid was covered with a piece of bolting silk (of 112 mesh to the linear inch) the margin of which was securely cemented to the

rim of the lid. This allowed free ventilation of the cell while preventing the escape of acari.

These cells were cemented on to a circular shaved patch of the sheep's skin. A cement, consisting of 10 parts of beeswax to 90 parts of resin, was found to be most satisfactory for attaching the cells. The cement was melted in a shallow glass dish on a water bath. The flange of the cell was brought in contact with the surface of the molten cement and then applied to the skin. The best site for the attachment of the cell was found to be within a

FIG. 6.
Sheep under examination on special examination table.

rectangular area on the dorso-lateral region, extending from two inches behind the point of the shoulder to about the tenth rib, and from about two inches from the spine down to the middle of the rib-length. In this area the skin surface was conveniently flat and, moreover, the sheep was not able to interfere with the attached cells. After the cell was fixed to the skin it was made more secure by tying two or three pairs of strands of the surrounding wool across the top of the cell.

In keeping acari in cells it was found necessary, owing to the growth of wool and the formation of crusts, to transfer them every three or four days to cells on a new patch of skin or on to the same patch after cleansing and re-shaving. In all the experiments recorded in this paper this transference was effected with a mounted needle without any damage to the acari. Where observations on

FIG. 7.
Examination of acari in a cell *in situ* on the sheep's back.

FIG. 8.
A latent case of sheep scab (Sheep No. 12), August 8th, 1936.

the hatching of eggs were made the cells were left in position long enough to ensure that neither eggs nor larvæ were overlooked. In order to facilitate the observations of the acari in cells a special table (Fig. 6) was made. The curved and slotted top was found to reduce considerably the discomfort of the sheep when strapped down for prolonged periods. A Greenhoff binocular microscope, on Universal stand (Zeiss stand XB), was used for observing the acari.

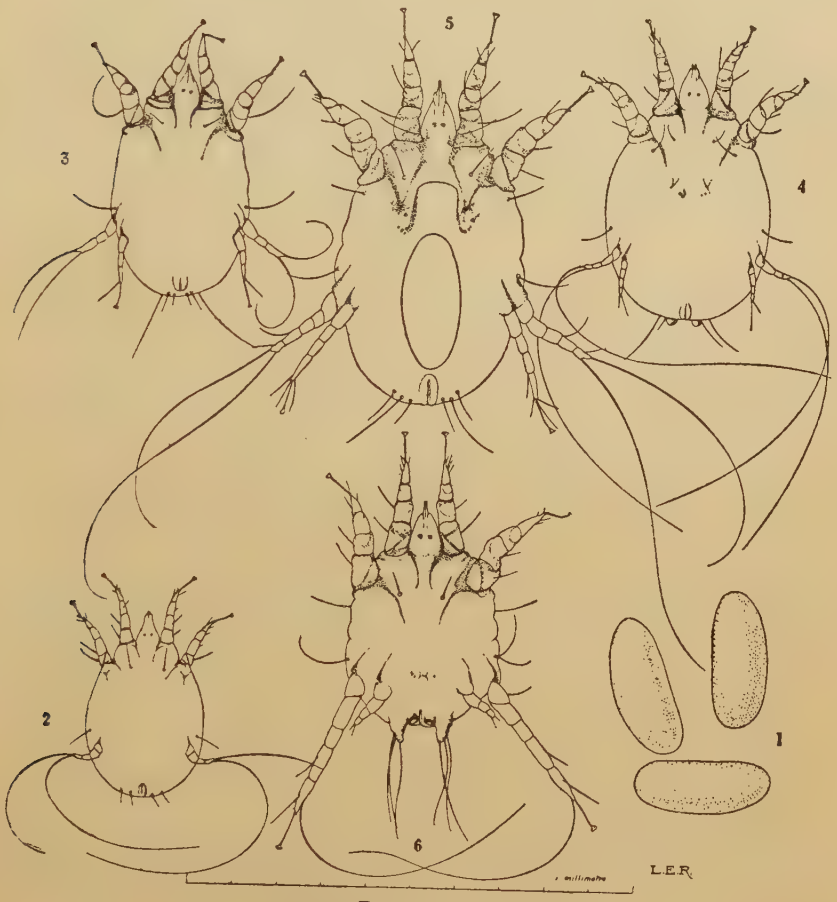


PLATE I.

*Psoroptes communis* var. *ovis*. Life stages.

1. Egg. 2. Larva. 3. Nymph. 4. Pubescent female. 5. Ovigerous female. 6. Male.

(All the figures are magnified to the same scale; the relative sizes are, therefore, comparable.)

The life-history has been observed by placing a copulating pair in a cell and, after the female had moulted to the ovigerous stage, collecting its eggs as soon as laid and observing the various stages and their intervals until the resultant females laid their first eggs.

### (2) *Experimental Data.*

(*a*) *The Egg.*—The egg of *Psoroptes communis* var. *ovis* (Plate I, 1) when newly laid is oval or slightly sausage-shaped, opaque and glistening white. In cells the eggs are laid close to where the female has fed. After the first 24 hours the egg becomes almost transparent and shortly before hatching a brownish area may be seen at one end, due to the coloured chitinous foreparts of the larva showing through the shell.

In cells on the sheep's skin, the egg usually takes three days to hatch. The shortest time observed was two days. Shilston (1915)[10] states that in South Africa " when close to the sheep's skin, the eggs hatch at intervals of two days from the time of laying and usually no egg remains unhatched at the end of three days."

Table I records observations on 126 eggs. It will be seen that the three-day interval occurs in 93 instances and that the average is 2·7 days.

Table I.

| *First Appearance of Egg.* | *First Appearance of Larvae.* | *Days.* | *Number of Larvae.* | *Remarks.* |
|---|---|---|---|---|
| 6. 1.33 | 9. 1.33 | 3 | 2 | |
| 26. 1.33 | 29. 1.33 | 3 | 2 | |
| 27. 1.33 | 29. 1.33 | 2 | 1 | |
| 6. 2.33 | 9. 2.33 | 3 | 2 | In same cell. |
| 7. 2.33 | 9. 2.33 | 2 | 2 | |
| 25. 4.33 | 27. 4.33 | 2 | 1 | |
| 26. 4.33 | 28. 4.33 | 2 | 7 | Same colony. 16 ovigerous females |
| 26. 4.33 | 29. 4.33 | 3 | 32 | |
| 19. 5.33 | 22. 5.33 | 3 | 4 | |
| 28. 6.33 | 30. 6.33 | 2 | 2 | |
| 2. 1.34 | 4. 1.34 | 2 | 2 | Same colony of 10 ovigerous females. Placed in cell on 1.1.34. 19 eggs found on 2.1.34. |
| 2. 1.34 | 5. 1.34 | 3 | 17 | |
| 3. 1.34 | 5. 1.34 | 2 | 11 | |
| 24.10.33 | 27.10.33 | 3 | 5 | |
| 13.12.33 | 16.12.33 | 3 | 2 | |
| 4 2.34 | 7. 2.34 | 3 | 2 | |
| 23. 2.34 | 25. 2.34 | 2 | 7 | |
| 12. 3.34 | 15. 3.34 | 3 | 3 | |
| 10. 7.34 | 13. 7.34 | 3 | 1 | |
| 12. 7.34 | 15. 7.34 | 3 | 14 | |
| 3. 8.34 | 6. 8.34 | 3 | 1 | |
| 17. 8.34 | 20. 8.34 | 3 | 6 | |

Average hatching period of egg in days = 2·7.

(*b*) *The Larva.* — The larva (Plate I, 2) has three pairs of legs, the first two of which bear ambulatory suckers. When newly hatched, the larva is very small and almost transparent, except for the capitulum and the legs, which at first are of a pale brown colour but later develop into a deeper brown. The larva is very active and begins to feed immediately after hatching and the typical vesicle is produced. The first 24 to 36 hours of the larval stage are spent in feeding. Whilst feeding, the larva

increases in size and assumes an opaque white appearance. In the majority of cases the larval phase lasts for two days, although in some instances it is prolonged to three days. For the last twelve to 24 hours of this phase the larva ceases to feed and becomes quiescent before ecdysis takes place.

In the moulting acarus a characteristic position of the body is assumed. The first two pairs of legs are stretched out in front, closely ranged against the capitulum; whereas in the dead acarus the first two pairs of legs are curled up under the body. This moulting position is characteristic of all the stages of the mite. The body of the moulting acarus also presents a typical appearance. The posterior four-fifths of the body has the normal opaque white colour, whilst the anterior fifth behind the capitulum appears almost transparent. When the specimens are mounted and cleared, the reason is apparent. The newly-formed acarus, *i.e.*, the nymph, does not completely fill the larval skin, the anterior portion of which is empty. This appearance has been observed in moulting acari of all stages.

Table II is a record of observations on 66 larvæ. The two-day interval occurs in 56 instances and the average is 2·2 days.

Table II.

| *First Appearance of Larvæ.* | *First Appearance of Nymph.* | *Days.* | *Number of Larvæ.* | *Remarks.* |
|---|---|---|---|---|
| 9. 1.33 | 12. 1.33 | 3 | 1 | |
| 10. 1.33 | 12. 1.33 | 2 | 1 | |
| 18. 1.33 | 20. 1.33 | 2 | 2 | |
| 19. 2.33 | 21. 2.33 | 2 | 3 | |
| 20. 2.33 | 22. 2.33 | 2 | 2 | |
| 4. 3.33 | 7. 3.33 | 3 | 1 | |
| 14. 3.33 | 17. 3.33 | 3 | 4 | |
| 27. 4.33 | 29. 4.33 | 2 | 10 | |
| 22. 5.33 | 24. 5.33 | 2 | 4 | |
| 27.10.33 | 30.10.33 | 3 | 5 | |
| 30.10.33 | 1.11.33 | 2 | 5 | |
| 15.12.33 | 18.12.33 | 3 | 1 | |
| 5. 1.34 | 7. 1.34 | 2 | 11 | |
| 6. 1.34 | 8. 1.34 | 2 | 10 | |
| 7. 2.34 | 9. 2.34 | 2 | 2 | |
| 25. 2.34 | 28. 2.34 | 3 | 2 | |
| 21. 8.34 | 23. 8.34 | 2 | 2 | |

Average life of larva in days = 2·2.

(*c*) *The Nymph.*—The nymphal or third stage is distinguished from the larva by the presence of four pairs of legs (Plate I, Fig. 3). Ambulatory suckers are present on the first two and fourth pairs of legs. The nymph, apart from the smaller size and the absence of the vulva, resembles the ovigerous female in its appearance.

After escaping from the larval skin the nymph feeds and grows for 24 hours. It then enters a quiescent period lasting from

24 to 36 hours, after which the pubescent female emerges. If the nymphs are destined to become males the nymphal stage is prolonged. The feeding period usually lasts for 48 hours and the quiescent stage 72 hours. The copulatory tubercles of the pubescent female are often visible through the nymphal skin before moulting is completed.

Table III records the observations on 34 nymphs which developed into pubescent females, and Table IV four nymphs which developed into males. The averages are 2·3 days and five days respectively.

Table III.

| *First Appearance of Nymph.* | *First Appearance of Pubescent Female.* | *Days.* | *Number of Nymphs.* | *Remarks.* |
|---|---|---|---|---|
| 20. 1.33 | 23. 1.33 | 3 | 2 | |
| 1. 2.33 | 3. 2.33 | 2 | 1 | |
| 21. 2.33 | 23. 2.33 | 2 | 2 | } Same colony. |
| 21. 2.33 | 24. 2.33 | 3 | 2 | |
| 22. 2.33 | 25. 2.33 | 3 | 2 | } Same colony. |
| 23. 2.33 | 25. 2.33 | 2 | 2 | |
| 7. 3.33 | 9. 3.33 | 2 | 5 | } Separate colonies. |
| 7. 3.33 | 9. 3.33 | 2 | 4 | |
| 1. 5.33 | 3. 5.33 | 2 | 2 | |
| 23. 5.33 | 25. 5.33 | 2 | 4 | |
| 30.10.33 | 1.11.33 | 2 | 4 | |
| 19.12.33 | 21.12.33 | 2 | 1 | |
| 8. 1.34 | 10. 1.34 | 2 | 2 | |
| 28. 2.34 | 2. 3.34 | 2 | 1 | |

Average life of nymph (female) in days = 2·3.

Table IV.

| *First Appearance of Nymph.* | *First Appearance of Male.* | *Days.* | *Number of Nymphs.* | *Remarks.* |
|---|---|---|---|---|
| 23. 2.33 | 28. 2.33 | 5 | 2 | 2 males out of 6 nymphs. |
| 27.10.33 | 1.11.33 | 5 | 1 | 1 male out of 5 nymphs. |
| 29.10.33 | 3.11.33 | 5 | 1 | „ „ |

Average life of nymph (male) in days = 5.

(*d*) *The Pubescent Female.*—The pubescent female has four pairs of legs (Plate I, Fig. 4), the first two of which bear ambulatory suckers. The chief distinguishing feature is a pair of copulatory tubercles, situated one on either side of the cloaca at the hinder margin of the body.

The pubescent female feeds for a short time and, if a male be present, fertilisation then takes place. In copulation the tubercles of the female are inserted into the copulatory suckers of the male; the capitula pointing in opposite directions. The females may feed during coitus. In cells when there is an equal or greater number of males than females the pairs remain coupled for 48

hours, although during the last 24 hours the female is actually undergoing its moult. If the males are fewer than the females the time of attachment may be reduced, but the female does not moult any sooner. On one occasion a male was put in a cell with five pubescent females. No observations were made during the next two days, but on the third day all the females had moulted to the ovigerous stage and in due course laid their eggs. On no occasion has a pubescent female been found to moult to the ovigerous stage in the absence of a male. How long a female may remain in the pubescent stage without fertilisation has not so far been observed. In a natural infestation it is probable that the copulatory period is quite short, as there are always fewer males than females. When coupled pairs are first put in a cell, or in the presence of artificial light, they show considerable activity. The male is always, however, the more energetic partner. Even when the female is active and not moulting she has to travel along behind him, and when she is moulting he finds her an easy burden. The male appears to attach himself firmly, as the writer has on more than one occasion observed males still carrying the moulted skins of the females around with them.

The pubescent stage usually lasts just over two days, but may be as long as four days. In Table V are given the records of 25 pubescent females. The average duration is 2·2 days.

Table V.

| *First Appearance of Pubescent Female.* | *First Appearance of Ovigerous Female.* | *Days.* | *Number of Females.* | *Remarks.* |
|---|---|---|---|---|
| 2. 2.33 | 5. 2.33 | 3 | 1 | |
| 23. 2.33 | 25. 2.33 | 2 | 1 | |
| 25. 2.33 | 27. 2.33 | 2 | 2 | } Separate cells. |
| 25. 2.33 | 28. 2.33 | 3 | 1 | |
| 10. 3.33 | 12. 3.33 | 2 | 1 | |
| 3. 5.33 | 5. 5.33 | 2 | 3 | |
| 15. 5.33 | 17. 5.33 | 2 | 4 | |
| 2.10.33 | 4.10.33 | 2 | 2 | |
| 1.11.33 | 3.11.33 | 2 | 2 | |
| 3.11.33 | 5.11.33 | 2 | 3 | |
| 16.11.33 | 20.11.33 | 4 | 1 | |
| 8.12.33 | 10.12.33 | 2 | 1 | |
| 8.12.33 | 12.12.33 | 4 | 1 | |
| 21.12.33 | 23.12.33 | 2 | 1 | |
| 9. 1.34 | 11. 1.34 | 2 | 1 | |

Average life of pubescent female = 2·2 days.

(*e*) *The Male.*—The male (Plate I, Fig. 6) has four pairs of legs, the third of which is very long and the fourth pair very short. Ambulatory suckers are present on the first three pairs of legs. The male is readily distinguished by the pair of posterior processes each bearing three large bristles. On the

ventral surface of the body, at the base of each process, on either side of the anus, is a copulatory sucker.

From nymphs of the same age, the males emerge from two and a half to three days after the pubescent females, or about seven to eight days after the hatching of the egg. The proportion of males to females, calculated from the data in Table VI, is in the ratio of 2 : 7·5. These observations are, however, very limited. Gerlach (1857)[5] gives the proportion as one to five and Shilston (1915)[10] as one to three or occasionally four.

Table VI.

| *Nymphs.* | *Males.* | *Pubescent Females.* | *Ratio.* |
|---|---|---|---|
| 6 | 2 | 4 | 1 : 2 |
| 5 | 1 | 4 | 1 : 4 |
| 5 | 1 | 4 | 1 : 4 |
| 6 | 1 | 5 | 1 : 5 |
| Total ... 22 | 5 | 17 | Approx. 2 : 7·5 |

The longevity of the male appears to be approximately one month. It is not known how many times copulation may take place during its life-time, as in most of the observations in cells the males have only been in contact with a limited number of pubescent females. From the writer's observations it would appear that the males search actively for the females and copulate freely. In one cell a male was found to fertilise three females in 48 hours and, as mentioned previously, another male fertilised five females in two days. All the females in due course developed into ovigerous females and laid eggs.

(*f*) *The Ovigerous Female.*—The ovigerous female (Plate I, Fig. 5) is easily distinguished by its relatively large size. It has four pairs of legs, of which the first two and the fourth carry ambulatory suckers. The chief distinguishing feature of the ovigerous female is the presence of the thoracic vulva, situated between the coxæ of the second pair of legs.

The actual ecdysis of the ovigerous female is of relatively short duration. During one observation a male and a pubescent female in moult were found coupled in a cell. In order to allow of the cell being changed they were transferred to a dish while the sheep's skin was being re-shaved. Immediately on being replaced in the new cell the ovigerous female emerged from the moulted skin, although an hour previously there had been no sign of ecdysis. The old skin was ruptured transversely across the posterior dorsal surface and from the middle of this line to the capitulum, the rupture making a T-shaped opening.

The ovigerous female soon commences to feed and feeding continues for one to two days (Table VII). It would appear to

ingest the bulk of its nutritive material in its first engorgement although it may also feed again in the intervals of oviposition. Immediately after engorgement oviposition commences. There is a considerable variation in the longevity of the ovigerous female and the rate of egg-laying. In Tables VIII (*a*), VIII (*b*) and VIII (*c*) are recorded observations on the periods of oviposition of a number of ovigerous females.

Table VII.

| *First Appearance of Ovigerous Female.* | *First Egg Observed.* | *Days.* | *Number of Females.* | *Remarks.* |
|---|---|---|---|---|
| 25. 1.33 | 26. 1.33 | 1 | 1 | |
| 4. 2.33 | 5. 2.33 | 1 | 1 | |
| 25. 2.33 | 27. 2.33 | 2 | 1 | |
| 28. 2.33 | 29. 2.33 | 1 | 1 | |
| 5. 5.33 | 6. 5.33 | 1 | 1 | |
| 26. 6.33 | 28. 6.33 | 2 | 2 | |
| 4.10.33 | 5.10.33 | 1 | 2 | |
| 12.12.33 | 13.12.33 | 1 | 1 | |
| 3. 1.34 | 4. 1.34 | 1 | 1 | |
| 3. 1.34 | 5. 1.34 | 2 | 2 | |

Pre-oviposition period in days = 1·3.

In Table VIII (*a*), in order to reach a comparable figure as to total number of eggs laid by the two females which survived the longest (18.12.33), deductions of 10, 42 and 34 eggs have been made, to allow for those which were laid by the females which died on 10.11.33, 20.11.33 and 1.12.33 respectively. Further, it was not until 6.11.33 that all the females reached the ovigerous stage, by which date seven eggs had been laid. These seven eggs have also been deducted from the total, and 6.11.33 taken as the date the two females which died on 18.12.33 reached the ovigerous stage. On this calculation the two females lived 42 days, during the last 13 days of which oviposition had ceased. They, therefore, laid 35 eggs each in 29 days at a daily average of 1·2 eggs per day.

In Table VIII (*b*) three females laid 119 eggs in eleven days, which is equivalent to 39·7 eggs each and a daily average of 3·6 eggs per day, with a maximum of four eggs per day between 16.3.34 and 19.3.34. Of these three females, one lived 11 days and the other two 15 days. In Table VIII (*c*) one female lived eleven days, during seven days of which it laid 39 eggs at a daily average of 5·59 eggs per day and a maximum of seven eggs on 18.11.33. In these three experiments, either the females were transferred to a new cell or the eggs removed after each count. Where the females were removed, the cell was left in position for a sufficiently long time for any eggs which had been overlooked to hatch out into larvæ.

TABLE VIII (*a*).

| *Date.* | *Ovigerous Females.* | *Eggs Laid.* | *Temperature.* | | *Rainfall in Inches per Day.* | *Remarks.* |
|---|---|---|---|---|---|---|
| | | | *Maximum.* | *Minimum.* | | |
| | | | °F. | °F. | | |
| 3.11.33 | — | — | 44 | 33 | Nil. | 4 pubescent ♀♀<br>2 nymphs ♂♂ added. |
| 4.11.33 | — | — | 46 | 34 | „ | No observations. |
| 5.11.33 | — | — | 43 | 33 | 0·02 | |
| 6.11.33 | 3 | 7 | 53 | 33 | 0·05 | 3 ovigerous ♀♀<br>2 pubescent ♀♀<br>1 moulting ♀ |
| 7.11.33 | 6 | 16 | 49 | 42 | Nil. | All 6 ovigerous ♀♀ |
| 8.11.33 | 6 | 18 | 46 | 40 | 0·14 | |
| 9.11.33 | 6 | 14 | 45 | 38 | 0·02 | |
| 10.11.33 | 6 | 14 | 37 | 32 | Nil. | 1 ♀ died. |
| 11.11.33 | 5 | — | 43 | 29 | „ | No observations. |
| 12.11.33 | 5 | — | 42 | 26 | 0·09 | |
| 13.11.33 | 5 | — | 43 | 27 | 0·37 | |
| 14.11.33 | 5 | 14 | 42 | 31 | 0·34 | |
| 15.11.33 | 5 | — | 38 | 29 | 0·01 | No observation. |
| 16.11.33 | 5 | 12 | 39 | 37 | 0·03 | |
| 17.11.33 | 5 | 14 | 43 | 38 | 0·01 | |
| 18.11.33 | 5 | — | 49 | 38 | 0·02 | No observation. |
| 19.11.33 | 5 | — | 55 | 44 | 0·01 | |
| 20.11.33 | 5 | 13 | 40 | 41 | Nil. | 2 ♀♀ died. |
| 21.11.33 | 3 | 6 | 40 | 41 | „ | |
| 22.11.33 | 3 | 5 | 42 | 38 | „ | |
| 23.11.33 | 3 | 8 | 42 | 33 | 0·08 | |
| 24.11.33 | 3 | 6 | 38 | 33 | 0·04 | |
| 25.11.33 | 3 | — | 34 | 30 | Nil. | No observation. |
| 26.11.33 | 3 | — | 38 | 31 | „ | |
| 27.11.33 | 3 | 12 | 35 | 29 | „ | |
| 28.11.33 | 3 | 0 | 33 | 33 | „ | |
| 29.11.33 | 3 | 0 | 35 | 33 | „ | |
| 30.11.33 | 3 | — | 33 | 28 | „ | No observation. |
| 1.12.33 | 3 | 2 | 33 | 29 | „ | 1 ♀ died. |
| 2.12.33 | 3 | — | 33 | 29 | „ | No observation. |
| 3.12.33 | 3 | — | 31 | 32 | „ | |
| 4.12.33 | 3 | 2 | 31 | 25 | „ | |
| 5.12.33 | 2 | — | 28 | 25 | „ | |
| 6.12.33 | 2 | 0 | 30 | 20 | „ | |
| 7.12.33 | 2 | 0 | 34 | 20 | 0·01 | |
| 8.12.33 | 2 | — | 32 | 31 | Nil. | No observation. |
| 9.12.33 | 2 | — | 28 | 22 | „ | |
| 10.12.33 | 2 | — | 31 | 21 | „ | |
| 11.12.33 | 2 | 0 | 32 | 21 | „ | |
| 12.12.33 | 2 | 0 | 31 | 27 | 0·02 | |
| 13.12.33 | 2 | 0 | 30 | 27 | Nil. | |
| 14.12.33 | 2 | 0 | 32 | 21 | 0·03 | |
| 15.12.33 | 2 | 0 | 36 | 21 | Nil. | |
| 16.12.33 | 2 | — | 32 | 30 | „ | No observation. |
| 17.12.33 | 2 | — | 32 | 27 | „ | |
| 18.12.33 | 2 | 0 | 37 | 27 | „ | 2 ovigerous ♀♀ died. |

Since these observations indicate that there is some correlation between the duration and rate of oviposition and frosty weather, the maximum and minimum temperatures and the rainfall at the Cooper Field Research Station, Little Gaddesden, where the investigations were carried out, have been included in Tables VIII (*a*), VIII (*b*) and VIII (*c*).

TABLE VIII (*b*).

| Date. | Number of Ovigerous Females. | Number of Eggs. | Maximum Temperature for 24 Hours. °F. | Minimum Temperature for 24 Hours. °F. | Rainfall for 24 Hours in Inches. | Remarks. |
|---|---|---|---|---|---|---|
| 9.3.34 | — | 0 | 50 | 26 | 0·10 | 3 pubescent ♀♀ *in copula*. |
| 10.3.34 | 3 | — | 52 | 30 | 0·30 | No observation. |
| 11.3.34 | 3 | — | 54 | 31 | 0·25 | |
| 12.3.34 | 3 | — | 54 | 28 | 0·19 | |
| 13.3.34 | 3 | 9 | 36 | 34 | 0·07 | |
| 14.3.34 | 3 | 11 | 46 | 30 | 0·39 | |
| 15.3.34 | 3 | 22 | 38 | 30 | 0·09 | Examination late in day, probably 30 hours' interval. |
| 16.3.34 | 3 | 13 | 49 | 32 | 0·20 | |
| 17.3.34 | 3 | — | 49 | 30 | Nil. | No observation. |
| 18.3.34 | 3 | — | 44 | 27 | ,, | |
| 19.3.34 | 3 | 36 | 45 | 26 | 0·25 | 3 days at 4 eggs per day per female. |
| 20.3.34 | 3 | 8 | 40 | 35 | 0·16 | |
| 21.3.34 | 3 | 10 | 39 | 36 | 0·09 | |
| 22.3.34 | 3 | — | 46 | 25 | Nil. | No observation. |
| 23.3.34 | 3 | 10 | 46 | 30 | ,, | 1 ♀ died. |
| 24.3.34 | 3 | — | 49 | 27 | 0·08 | No observation. |
| 25.3.34 | 3 | — | 55 | 41 | — | |
| 26.3.34 | 2 | 0 | 55 | 30 | Nil. | ♀♀ died. |

TABLE VIII (*c*).

| Date. | Number of Ovigerous Females. | Number of Eggs. | Maximum Temperature for 24 Hours. °F. | Minimum Temperature for 24 Hours. °F. | Rainfall for 24 Hours in Inches. | Remarks. |
|---|---|---|---|---|---|---|
| 14.2.33 | 1 | 0 | 42 | 26 | Nil. | ♀ moulted to ovigerous stage. |
| 15.2.33 | 1 | 0 | 38 | 27 | ,, | |
| 16.2.33 | 1 | 5 | 42 | 29 | 0·03 | |
| 17.2.33 | 1 | 6 | 41 | 29 | 0·02 | |
| 18.2.33 | 1 | 7 | 33 | 26 | 0·11 | |
| 19.2.33 | 1 | — | 30 | 28 | 0·09 | |
| 20.2.33 | 1 | 11 | 39 | 21 | Nil. | |
| 21.2.33 | 1 | 5 | 40 | 22 | 0·10 | |
| 22.2.33 | 1 | 5 | 33 | 25 | Nil. | |
| 23.2.33 | 1 | — | 34 | 22 | ,, | |
| 24.2.33 | 1 | — | 32 | 17 | 0·29 | |
| 25.2.33 | 1 | 0 | 34 | 22 | 0·79 | ♀ died. |

This relationship between temperature and oviposition is more clearly indicated in Table IX, which gives the average maximum and minimum temperatures and the total and average rainfall for each experiment from the moulting of the ovigerous female to the cessation of egg-laying. It is of interest to note that the lower the average minimum temperature (frosty nights) the greater is the output of eggs per day and the shorter is the life of the ovigerous

female. Rainfall does not appear to have much influence on oviposition. Shilston (1915)[10] cites an instance where on the advent of heavy rain, following a period of drought, an ovigerous female began egg-laying at the rate of seven eggs per day, although previously it had only laid three eggs in ten days. Unfortunately he does not give any records of the temperature. It would be unwise, however, to compare the conditions in Natal and Great Britain, since in this country, even during fine weather, we do not experience the exceptionally dry conditions of Natal. The influence of climatic conditions is further discussed in the section headed "The Clinical Aspect of Sheep Scab."

Table IX.

| *Period.* | *Eggs Laid per Day.* | *Longevity of Female in Days.* | *Temperature.* | | *Rainfall Total and Average in Inches.* | *Remarks.* |
|---|---|---|---|---|---|---|
| | | | *Average Maximum.* | *Average Minimum.* | | |
| *Table VIII (a)* | | | | | | |
| 6.11.33 to 4.12.33 | 1·2–1·3 | 42 | 40·7° F. | 33·8° F. | T. 1·21<br>Av. 0·04 | Minimum temperature only 4 times below 32° F.<br>Highest maximum temperature 53° F. |
| *Table VIII (b)* | | | | | | |
| 9.3.34 to 23.3.34 | 3·6 | 13–15 | 45·9° F. | 30° F. | T. 2·09<br>Av. 0·139 | Minimum temperature only 3 times above 32° F.<br>Highest maximum temperature 50° F. |
| *Table VIII (c)* | | | | | | |
| 14.2.33 to 22.2.33 | 5·59 | 11 | 33·5° F. | 25·9° F. | T. 0·35<br>Av. 0·035 | Minimum temperature never above 32° F.<br>Highest maximum temperature 42° F. |

Gerlach (1857)[5] gives the total number of eggs laid by an ovigerous female as 15. Stockman and Berry (1913)[9] give the total eggs as 15 to 30, and the duration of the life of the ovigerous female as eight days. Shilston (1915)[10] found considerable variation, but states that "under favourable conditions the number may exceed 90 eggs per female. The life of the ovigerous female on sheep is 30 to 40 days."

(*g*) *The Complete Life-History.*—In Table X are given five complete life-cycles which were followed through by the cell method, from egg to egg. In two instances the life-cycle covered eleven days, in one instance twelve days, and in another ten days. The fifth took ten days to reach the ovigerous stage, but the laying of the first egg was not observed. The most frequent life-cycle is eleven days. Below is given a summary of Tables I, II, III, V and VII, being the average duration of each stage.

| | |
|---|---|
| Table I —egg ... ... ... ... | 2·7 days |
| Table II —larva ... ... ... ... | 2·2 ,, |
| Table III —nymph ... ... ... ... | 2·3 ,, |
| Table V —pubescent female ... ... | 2·2 ,, |
| Table VII—ovigerous female to first egg ... | 1·3 ,, |
| *Total in days* ... ... | 10·7 |

Table X.

| *Date.* | *Number* 1. | *Number* 2. | *Number* 3. | *Number* 4. | *Number* 5. |
|---|---|---|---|---|---|
| Appearance of egg ... | 16.2.33 | 17.2.33 | 26.4.33 | 24.10.33 | 13.12.33 |
| Appearance of larva ... | 19.2.33 | 20.2.33 | 28.4.33 | 27.10.33 | 16.12.33 |
| Appearance of nymph ... | 21.2.33 | 22.2.33 | 1.5.33 | 29.10.33 | 19.12.33 |
| Appearance of pubescent female ... ... ... | 23.2.33 | 25.2.33 | 3.5.33 | 1.10.33 | 21.12.33 |
| Appearance of male ... | | 28.2.33 | | | |
| Appearance of ovigerous female ... ... ... | 25.2.33 | 28.2.33 | 5.5.33 | Not observed | 23.12.33 |
| Appearance of egg ... | 27.2.33 | 1.3.33 | 6.5.33 | 4.11.33 | Not observed. |
| Total in days ... ... | 11 | 12 | 10 | 11 | Probably 11 or 12. |

It is interesting to compare the above results with the recorded observations of other writers. Gerlach (1857)[5] gives the life-cycle as 14 to 15 days. In one experiment he placed a coupled pair on a sheep. The male died and the female moulted to the ovigerous stage and laid her eggs. He then observed the first appearance of a second coupled pair, which occurred on the fifteenth day. He did not, however, appear to have been aware that males of the same generation require two and a half to three days longer than the females to develop. If all the acari on a sheep are of the same generation, the life-cycle is unduly prolonged. Stockman (1910)[7] quotes the classical life-cycle as twelve to 16 days. Shilston (1915)[10] gives the life-cycle as usually eleven days. In Table X the period from the first observed appearance of the larva to the egg-laying of the resultant female is eight days. The significance of these periods will be discussed later in the section "Bionomics in Relation to Control Measures." The periods covered in Table X are February, April, May, October and December.

One further life-history which occurred in July but which was not included in Table X as the first appearances of the different stages were not observed, is of interest. The sheep on which the observations were carried out was very slightly infected with scab.

13.7.34.—Eggs were laid by three ovigerous females. The females were removed from the cell.

15.7.34.—Larvæ were present in cell.

19.7.34.—Nymphs in moult were present in the cell. Males were added.

23.7.34.—Ovigerous females were present but no eggs were laid.

The females took ten days to reach the ovigerous stage, which conforms to the life-cycles in Table X.

From the whole of the foregoing observations there is no evidence to show that the life-cycle varies in the different seasons of the year.

*(To be continued.)*

## REVIEWS

**Histoire de la Médecine Vétérinaire.** Par E. Leclainche, Membre de l'Institut, Office du Livre-Toulouse, rue Alsace-Lorraine, 1936. Pp. 812. Copyright by the Author. All Rights, especially that of translation, reserved in all countries.

This learned work will everywhere be recognised as one of very exceptional merit. In the extent and interest of the information which it conveys on the history of veterinary medicine, it exceeds all previous works on the same subject.

The reader's interest is greatly enhanced at the outset by the fact that he is taken in imagination to the earliest discovered history of man himself, and shown how, in the slow evolution of civilisation, human beings gradually came to live in more or less close association with different species of wild animals, bred in confinement. This process of domestication must first have provided opportunities for man to observe and reflect on the deviations from the normal in his animals during life or after death, and to consider the possibility of extending to them methods of treatment or cure already applied to his own species. The treatment of disease in animals, was honoured and practised for eight centuries in the Greek and Roman Empires, but afterwards it declined and was forgotten and scorned for 1,400 years.

The two branches of medicine—human and animal—might have become fused if the Christian religion had not conquered Greece and Rome. The philosophy of Plato and the new religion were in accord in repelling as an outrage on Divinity the idea of giving the same care to men and animals. On the other hand, under the ancient philosophy in Asia life and its mystery were revered, and the Divine principle was believed to sanctify all living things. There, there was only one medicine, and the author suggests that it might have been the same everywhere to-day if the religion of Buddha had spread over the world, but, in order the better to affirm the absolute character of its dogmas, the Christian Church exaggerated its scorn for animals and went so far as to refuse them even its pity. Moreover, the politics of the Church aggravated the rigour of its doctrines. It was not only necessary to implant in the spirit of the faithful the abstract notion of a soul ; it was also necessary to premunise its members against the persisting influence of the older religions of the East.

Although thus annihilated in the Christian world by the interdict thrown on it, veterinary medicine rose again as soon as it escaped from the reprobation which had crushed it. Among the Arabs it refound its place by the side of human medicine ; under the ægis of a religion which opposed no obstacle to it, the art was taught in the great schools, and the two medicines, human and veterinary, were equally honoured. The influence thus exercised was so profound that it persisted after the departure of the conquerors, and the Catholic kings continued to accord the same solicitude as the Arabs to animal medicine.

It would be unfair to quote at greater length, but these passages have been selected from the preface and given almost literally to show the extraordinary interest of the matters dealt with in the book.

The first part of the work deals with the origin of the Schools. It has three chapters, the first of which deals with the information that has been gathered regarding the treatment of animals during what may be termed the dawn of civilisation. The second chapter covers the period of the Middle Ages, and the third the period of the Renaissance.

The second part forms three-fourths of the remainder, and, with the exception of about 100 pages, it is devoted to the separate history of each of modern Veterinary Schools or Colleges, sixty-nine in number.

There follows a short account of what are termed International Organisations, including the International Veterinary Congresses, the International Office for Epizootics, the Conferences held at Geneva and Regional Conferences.

Three final short chapters deal respectively with the contagious diseases of animals in the ninteenth and twentieth centuries ; veterinary medicine since the foundation of the Schools ; and the veterinary profession from the eighteenth to the twentieth century.

The author appears to have consulted all the available sources of information on the history of veterinary medicine, and one of the merits of the book is that it is richly documented.

No veterinary work that has appeared during the present century deserves greater praise than this, and probably none is capable of having an equal effect in raising the pride of veterinary surgeons in the utility and nobility of their profession, and in the progress it has made in the last 100 years in spite of much neglect and actual discouragement. A perusal of its pages suggests the thought that it would be a wise proceeding if those who are responsible for the curriculum in the veterinary schools throughout the world were to take steps to ensure that no student is granted a diploma to practise without having received such instruction in the history of his profession as is given in this great work. The foundation of lectureships on that subject is greatly to be desired.

The preparation of the work must have been spread over many years and imposed a severe strain on the health and energy of the author, but no doubt it has been a labour of love. It will certainly increase the honour in which he was already held for his many valuable contributions to veterinary literature and research.

**Handbuch der Anatomie des Hundes.** By Hermann Baum und Otto Zietzschmann. Second Completely Revised Edition of the Anatomie des Hundes von Ellenberger und Baum. Berlin : Paul Parey, 1936. Pp. 250 with 180 Text Figures. Price, bound, Rm. 26.

The fact that in the large towns, since the introduction of motor traction, the equine population has sunk to insignificant numbers while dogs have greatly increased, has given greater importance to the latter species from the veterinary practitioner's point of view. It has, therefore, become essential that students should have for their guidance in the practical study of the anatomy of the dog the help of a good text book, and especially of one with good illustrations. The present work, which deals only with bones and muscles, can be strongly recommended.

The illustrations are excellent, and display practically every bone and muscle *in situ*. The descriptions of each object given in the text are clear and sufficiently detailed.

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